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Identification of *E. coli* collected from water of various farms from Akola region

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Abstract
The present study was to identify the *E. coli* collected from Akola district. Twenty one water samples were collected for morphological, bacteriological and biochemical investigations for isolation and identification of *E. coli*. The samples were cultured on nutrient broth and after culturing the same samples transferred to specific media EMB & MC agar. Biochemical properties of the isolates were considered and response in TSI test incline was likewise watched. The samples which give particular morphological and staining characteristic were chosen for the further biochemical examination. From the present study it was concluded that the general prevalence of *E. coli* was recorded as 66.66% from the poultry farms. The outcomes recommended that water samples collected from the various farms were shown to be a significant wellspring of *E. coli* infections in the field conditions. The presence of *E. coli* at the recorded rate in water samples poses significant alarming for the public health issue if not maintain proper hygienic steps taken place.

Keywords: prevalence, *E. coli*, water, broilers

Introduction
Colibacillosis is an infection of poultry which causes serious economic losses to all the poultry industry and various farmers worldwide. Akola is located in a Vidharbha region of Maharashtra state with a considerable number of household rearing the broiler birds. About more than 65% of the population is consuming non-vegetarian food and as per the Indian Council Medical Research recommendation meat consumption is of 30g/person/day but meat availability in India are only about 15g/person/day [14]. The headway of the poultry industry in Akola was hindered by various limitations, of which significant one is a flare-up of sickness causing about 30% mortality of chickens in consistently. The major etiological agents are the microorganisms, parasites, the board causes, ecological causes and lack of mineral and nutrients. The real reason for microorganisms is *Escherichia coli*, growth and so forth. Be that as it may, the pathogens spread from the chicken sully the litter, feed, water and also by the adjacent flying creatures. The quick development of the poultry business has brought about the generation of huge amounts of poultry squanders. These materials are then present in soil manures and nutritive substrates of the feeds for broilers [10]. However, wellspring of disease by pathogenic microorganisms, for example, *Listeria monocytogenes*, *Salmonella* and *Campylobacter spp* [12]. It is vital to know the predominance and circulation of various bacterial vegetation in poultry and its condition the same number of them might be potential pathogen for poultry. Such data is moreover required to take important activities for the counteractive action and control of infections brought about by bacterial pathogens. The present examination was embraced to indentify and portray the bacterial pathogens present in poultry water source as wellspring of potential contaminants in the poultry industry.

Materials and Methods
Sample collection
The aggregate of 21 water samples (500 mL) was collected from the various farms in and around the regions of Akola district. Water samples were collected aseptically and moved quickly into a sterile Petri dish. The samples were then conveyed to the laboratory in the Regional Disease Investigation Laboratory, Akola. These water samples were examined for different bacteriological and biochemical examination in the research centre. Nutrient Agar (NA) was utilized for culturing the organism collected from the various water samples before

performing the biochemical test. Eosin Methylene Blue agar medium was utilized to observe the development of *E. coli* [2]. MacConkeys medium was utilized for culturing of the specific organism under the family Enterobacteriaceae [10].

Media

Nutrient Broth, Motility Indole Urea medium, Nutrient Agar medium, Eosin Methylene Blue Agar, MacConkey's Agar medium, Triple Sugar Iron Agar test were obtained as powdered structure and prepared the media as per the standard protocol.

Reagents and Solution

Voges-Poskauer solution, Methyl Red, Gram's iodine, Crystal violet, Acetone alcohol, Safranine, Kovac's reagent, Phosphate Buffered Saline (PBS), Alpha naphthol solution, Potassium hydroxide solution and Ethyl alcohol (70% and 95%).

Inoculums preparations

Water sample 500mL were collected from the different farms of the Akola regions and mixed thoroughly. From the same samples 1 mL of water was taken and transferred this into 9 mL of Enterobacteriaceae Enrichment broth and incubated @ 37°C for 24 hrs under aerobic condition. After which a loopful of the enriched culture from Enterobacteriaceae Enrichment broth was streaked onto Eosin Methylene Blue agar and again incubated @37°C for 24 hrs under aerobic condition [1].

Sample enrichment

After collection of the water, samples were enriched in nutrient broth media and incubated at 37^0C for 12 hrs.

Bacterial isolation

After enrichment in Nutrient broth a few quantities of inoculums from Nutrient Broth was streaked onto MacConkey agar and Triple Sugar Iron agar. The inoculated plates were incubated at 37^0C for 12 hrs. After confirmation microbial culture on the specific media, various screening media such as Triple Sugar Iron agar was utilized for further processing. The test organism was cultured into Triple sugar iron agar by streaking method [13].

In triple sugar iron agar slant, if tube showed yellow color in few hrs then organism ferments only glucose. If the slant showed yellow color and it will persist for many days then organism ferments lactose this indicates acid production elevated. Organism able to produce gas by showing bubble formation. Appearance of gas, butt and slant yellow in color with the absence of precipitation black color in the butt leads positive indication for *E. coli* organism [13].

Bacterial identification

Gram's staining

The technique depicted by [4] for microscopic identification of *E. coli* colonies by Gram's stain. A, colony was taken up with the help of a bacteriological loop, prepare smeared on a glass slide and by gentle heating smear fixed. On the smear crystal violet solution added for two minutes and by tap water washed the smear. For a period of one minute add Lugol's iodine and again washed by tap water. For few seconds Acetone alcohol added further wash with running water. For a period of two minutes add safranine. Smear washed with tap water. Allow it for drying and go for the examination of smear under the microscope by using oil immersion.

Morphological characteristic

Morphological characteristic of colony such as size, shape, edge, surface texture, color, height and opacity developed after 24 hrs of incubation in different media were carefully observed.

Biochemical tests

E. coli isolated organism showing morphological colony characteristic on MacConkeys agar, Triple Sugar Iron test and Eosin Methylene Blue Agar were oppressed for biochemical tests such as MR-VP test, Indole tests, Sugar fermentation test [15].

Culturing of the organism

After the isolation of organism and the pure cultures were transferred periodically into a fresh medium (Sub culturing) to allow continuous growth and viability of microorganisms. The transfer was always subject to aseptic conditions to avoid contamination and kept in the refrigerator at 4°C for further use [10].

Results and Discussion

Isolation and identification of *E. coli*

Cultural prevalence of *E. coli in selected poultry farms.*

Table 1: *E. coli* predominance from isolated poultry farms.

S. No.	No. of farms	Type of sample	No. of samples	Positive for *E. coli*	Percentage
1	21	Water	21	14	66.66%

Isolated *E. coli* were identified from the selected samples after culturing on Nutrient Agar, Eosin Methylene Blue agar and MacConkey agar. The predominance of *E. coli* in the current study was 66.66% (Table 1). Each positive sample was treated as isolate. The predominance of *E. coli* in water sample was 66.66%.

Isolation of *E. coli* by various colony characteristic on agar

Table 2: Morphology, cultural and staining characteristics of isolated *E. coli.*

S. No.	Media used	Colony characteristics	Morphology (staining Characters)
1	Nutrient Agar	Grayish white, thick, large, moist, smooth, opaque or translucent discs	Gram -ve, straight, rod shape organism. Present singly or paired.
2	Eosin Methylene Blue Agar	Metallic sheen green color, rapid lactose fermented	
3	Mac Conkeys Agar	Dry, flat, pink colonies with a adjacent darker pink area of bile salts precipitation. Lactose fermented.	

Identification of *E. coli by* biochemical test

E. coli isolates fermented all the sugars present in the samples producing gas and acid. Color changes from red to yellow with production of gas and acid was observed by bubble formation in the Durhams tube.

Table 3: Biochemical Test

S. No.	Biochemical reaction against *E. coli*		Result
1	Lactose fermentation		+
2	Sucrose fermentation		+
3	Dextrose fermentation		+
4	Indole production		+
5	Mannitol fermentation		+
6	Voges Proskauer test		-
7	Methyl Red test		+
8	Triple sugar iron test	Gas	+
		H_2S	-
		Slant	Yellow
		Butt	Yellow

In the current study *E. coli* isolated organism rapidly fermented manitol, lactose, maltose, dextrose and sucrose with the production of gas and acid.

Results of Indole, Nitrate reduction, Catalase, Methyl red test of the *E. coli* isolates were positive as reported by [17].

In Gram's staining, the morphology of the isolated *E. Coli* bacteria showed Grayish white, thick, large, moist, smooth, opaque or translucent discs on nutrient agar, Metallic sheen green color with rapid lactose fermenting colonies on EMB agar and Dry, flat, pink colonies with a adjacent darker pink area of bile salts precipitation seen in MacConkeys agar which was supported by several authors [13].

It was found that the prevalence of *E. coli* infection present in the collected water samples from the various farms of Akola region (66.66%).

Hill *et al.* [9] reported the prevalence of *E. coli* was 60% from the use of the animal waste i.e effluent or lagoon sediment. Majowicz *et al.* [11] reported 64% of *E. coli* isolated from the various foods. Bodering *et al.* [5] reported 65% prevalence of *E. coli* isolated from five different farms of the town of N'Djamena and Doha in Chad. Xie *et al.* [16] reported that eighty samples of *E. coli* isolated from the Chicken and pork was 64% & 70% respectively. Alnahass *et al.* [3] reported that the *E. coli* prevalence was 63.3% and 86.6% isolated from the liver and intestinal samples respectively of poultry in Egypt. These findings were in close agreement with the present finding of the prevalence of *E. coli* 66.66% isolated from the water of various farms of Akola region. Similar finding also reported by the Ghanbarpour *et al.* [8], Islam *et al.* [10] and Adzitey *et al.* [1]. Davis *et al*. [6] reported that the *E. coli* prevalence were 87.6% and 90.7% from the various products of Chickens and turkey products respectively. Prevalence of *E. coli* was higher (71.80%) reported by Derakhshantar and Ghanbarpour [7] might be due to seasonal variation, environmental factors and food habit.

Conclusion

From the present study it was concluded that the water samples collected from the various sources from the farms were shown to be a significant wellspring of *E. coli* infections in the present field conditions. The presence of *E. coli* at the recorded rate (66.66%) in water samples poses significant alarming for the public health issue if not maintain proper hygienic steps taken place.

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