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ISOLATION OF PATHOGENIC BACTERIA FROM DRINKING WATER OF SCHOOLS' WELLS AND TANKS IN JALALABAD CITY OF NANGARHAR PROVINCE, AFGHANISTAN

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Abstract–Waterborne illnesses are prevalent and have caused significant loses to the habitants of Nangarhar province. This is due to the lack of knowledge about waterborne pathogens and inaccessibility to safe drinking water. Thus, the present study was conducted to isolate and identify *Shigella, Salmonella* and *E. coli* from drinking water of Jalalabad Government and Private schools. Collectively, 300 drinking water samples (150 from wells + 150 from water tanks) of 100 ml were collected from government and private schools, and subjected to microbiological tests for *E. coli*, *Shigella* and *Salmonella* isolation in selected culture medium followed by Grams' staining and biochemical confirmation tests. The results revealed that, the prevalence of *Shigella, Salmonella* and *E. coli* was 60%, 38%, and 20.7% in wells, and 63.3%, 34%, and 30.7% in water tanks, respectively. The results also depicted that the prevalence of *Shigella* was associated with deepness of well, and *Salmonella* and *E. coli* with distance of well from toilet and authority type of school ($p<0.01$). Moreover, the prevalence of *E. coli* and *Salmonella* were associated with type of tank and authority, and *Shigella* with type of tank ($p<0.01$), In conclusion, the drinking water of schools in Jalalabad city is not safe from microbiological point of view and the prevalence of the common waterborne pathogens is significantly associated with studied factors.

INTRODUCTION

Safe water is one of the most important felt needs in public health in the twenty first century (Sobsey and Bartram, 2003). Drinking water that is visually clear and colorless is acceptable. It should, however, be free of chemical toxins and dangerous microorganisms (Maheshwari, 2008). Humans, pests, ruminants, non-ruminants, and wild animals all have *E. coli* in their gastrointestinal tracts, where they are known to reside as commensals. Many distinct *Salmonella* serotypes can be found in humans, animals, and birds to variable degrees. All members of the genus have the potential to cause disease. Salmonella species which are found in water mainly originate from sewage and sewage effluents. *Salmonell*a counts in water are often significantly lower compared to other bacteria (Barrell *et al*., 2006). *Salmonella* spp., *V. cholera*, and *E. coli* are listed under zoonotic agents. The possible sources of these bacteria are humans, animals, and sewage. Contaminated water is a major source of bacterium transmission to human (Momtaz *et al*., 2013).

The presence of fecal coliform in water could indicate that the water has been contaminated with human or animal feces. Coliform bacteria should not be present in groundwater obtained from a properly constructed well. Direct discharge of waste from mammals and birds, as well as agricultural and human sources, can cause fecal coliform contamination of wells' water. Poor well construction or cracks, as well as runoff from wooded areas, pastures, feedlots, septic tanks, and sewage plants into streams or groundwater, could also cause the aforementioned contamination (Aboh *et al.,* 2015). Thus, monitoring the microbiological quality of drinking water on a regular basis is an important part of guaranteeing its safety (Pillai *et al.,* 1997).

To our knowledge, there is no information about waterborne pathogens in Nangarhar province. Thus, the present study recorded the observations on the pathogenic bacteria (*Salmonella, Shigella* and *E. coli*) isolated from drinking water of government and private schools in Jalalabad city in Nangarhar province, Afghanistan. Moreover, the present study also investigated the association of prevalence of aforementioned pathogens and deepness of wells, distance of wells from septic tanks and the types of tanks (plastics, Metallic and Concrete).

MATERIALS AND METHODS

Study Design

Totally 300 (150 from tube wells + 150 from water tanks) samples of drinking water were collected from (34) government and (34) private schools in Jalalabad city of Nangarhar province. The schools were selected randomly for sample collection, and a consent form was signed by head of school in order to give permission for sample collection.

Drinking Water Sample Collection

After letting the tap run for a minute, samples of 100 ml of drinking water were collected in a pre-sterilized borosilicate glass bottle. After that, the samples were appropriately labeled and transferred to the laboratory in ice packs for microbiological testing. The samples were examined within 8 hr after receiving to the laboratory (Baird, 2017).

Isolation and Identification of Pathogens

Three pathogens namely *Salmonella, Shigella* and *E. coli* were isolated in Xylose Lysine Desoxycholate agar (XLD agar), *Salmonella Shigella* agar (SS agar) and Eosin Methylene Blue agar (EMB agar), respectively. The pathogens were further identified by undertaking the respected biochemical examinations as reported by Da Silva *et al*. (2013).

Statistical Analysis

The collected data were analyzed by using SPSS V.23. The data were subjected to Chi-square test of independence in order to investigate the association of prevalence and influential factors. The association was considered significant when $p<0.05$. The results were reported as percentage.

RESULTS

The present study was designed to investigate the prevalence of *E. coli, Shigella* and *Salmonella* and associated factors in drinking water of private and government schools. In the present study, the prevalence of aforementioned microorganisms were studied in drinking water of tanks and wells, and then the associated factors like deepness of well, distance of well from toilet, type of tank and others were studied. The results are described as follow.

Drinking Water from Well

The data concerning prevalence of *E. coli, Shigella* and *Salmonella* in drinking water of wells, and associated factors like deepness, authority (private, government), and distance from toilet, are depicted in Table 1, Table 2 and Table 3 respectively. The results showed high prevalence for *Shigella* (60%) followed by *Salmonella* (38%) and *E. coli* (20.7%). The results also showed that prevalence for *Shigell*a was associated with deepness of well ($p<0\ 0.01$), but prevalence for *Salmonella* and *E. coli* were not associated with deepness of well ($p>0.05$). Moreover, the prevalence of *Salmonella* and *E. coli* were high significantly associated with distance of

Table 1. Prevalence and associated factors of *E. coli* in well drinking water.

Grouping Variable	Groups	Prevalence of *E. coli*				
		Positive	Negative	Total	Prevalence (%)	P-value
Deepness	Shallow	4	11	15	26.66	0.545
	Deep	27	108	135	20.00	
Distance of well from toilet	10-20m	3	29	32	3.38	0.005
	20-30m	1	24	25	4.00	
	>30m	27	66	93	29.03	
Type of Authority	Private	7	62	69	10.14	0.03
	Public	24	57	81	29.63	

Table 2. Prevalence and associated factors of *Shigella* in well drinking water.

Grouping Variable	Groups	Prevalence of *Shigella*				
		Positive	Negative	Total	Prevalence (%)	P-value
Deepness	Shallow	15	0	15	100.00	0.001
	Deep	75	60	135	55.55	
Distance of well from toilet	10-20m	20	12	32	62.50	0.821
	20-30m	16	9	25	64.00	
	>30m	54	39	93	58.06	
Type of Authority	Private	44	25	69	63.77	0.385
	Public	46	35	81	56.79	

Table 3. Prevalence and associated factors of *Salmonella* in well drinking water.

Grouping Variable	Groups	Prevalence of *Salmonella*				
		Positive	Negative	Total	Prevalence (%)	P-value
Deepness	Shallow	8	7	15	53.33	0.197
	Deep	49	86	135	36.30	
Distance of well from toilet	10-20m	2	30	32	6.25	0.000
	20-30m	8	17	25	32.00	
	>30m	47	46	93	50.54	
Type of Authority	Private	7	62	69	10.15	0.003
	Public	24	57	81	29.63	

Table 4. Prevalence and associated factors of *E. coli* in tank drinking water.

Grouping Variable	Groups	Prevalence of *E. coli*				
		Positive	Negative	Total	Prevalence (%)	P-value
Tank Type	Metallic	14	9	23	60.87	0.000
	Plastic	11	56	65	16.92	
	Concrete	21	39	60	35.00	
Type of Authority	Private	31	36	67	46.27	0.000
	Public	15	68	83	18.07	

Table 5. Prevalence and associated factors of *Shigella* in Tank drinking water.

Grouping Variable	Groups	Prevalence of *Shigella*				
		Positive	Negative	Total	Prevalence (%)	P-value
Tank Type	Metallic	13	10	23	56.52	0.734
	Plastic	44	23	67	65.67	
	Concrete	38	22	60	63.33	
Type of Authority	Private	60	23	83	72.29	0.009
	Public	35	32	67	52.24	

Table 6. Prevalence and associated factors of *Salmonella* in Tank drinking water.

Grouping Variable	Groups	Prevalence of *Salmonella*				
		Positive	Negative	Total	Prevalence (%)	P-value
Tank Type	Metallic	3	20	23	13.04	0.010
	Plastic	20	47	67	29.85	
	Concrete	28	32	60	46.66	
Type of Authority	Private	16	67	83	19.28	0.000
	Public	35	32	67	52.24	

well from toilet and authority type of school ($p<0.01$), but was not significantly associated with the prevalence of *Shigella* ($p>0.05$).

Drinking Water from Tank

The prevalence of *E. coli*, *Shigella* and *Salmonella* in drinking water of tanks, and associated factors like type of tank and type of authority are presented in Table 4, 5 and 6 respectively. Based on results, the high prevalence was recorded for *Shigella* (63.3%) followed by *Salmonella* (34%) and *E. coli* (30.7%). Furthermore, the results showed that type of tank and authority were highly associated with *E. coli* prevalence ($p<0.001$). In like manner, the type of authority was significantly associated with the prevalence of *Shigella* in tank drinking water ($p<0.01$), but was not with type of tank ($p>0.05$). Moreover, the prevalence of *Salmonella* was also significantly associated with both type of tank and authority ($p<0.01$).

DISCUSSION

Water is necessary for life on earth, but it may also act as an optimum medium for various pathogens. Every human being has the right to drink safe and healthy water. Unsafe drinking water and unsanitary circumstances enhance the risk of different health hazards like typhoid fever, shigellosis, and colibacilosis. According to the literatures, WHO estimated that 60% of all deaths due to diarrhea in low- and middle-income countries are attributable to inadequate drinking-water (35%), sanitation (31%) and hygiene (12%) (WHO, 2019). The purpose of this study was to look at the prevalence of *Shigella, Salmonella,* and *E. coli* in water, as well as their associated factors.

This study revealed that the prevalence of *Shigella* and *Salmonella* were more than *E. coli*. It means that in Afghanistan, most government and private schools drinking water were contaminated, particularly in Jalalabad city. Moreover, the type of water tank, type of authority of school, deepness of the well and the distance of well from the toilet were affecting factors the prevalence of pathogens in drinking water. The pathogens might be the main cause of water borne diseases in children schools. In likewise, several authors also isolated *E. coli*, *Shigella* and *Salmonella* from samples of water (Falcao *et al.,* 1993; Talukder *et al.,* 2013; Mian *et al.,* 2020). A study was conducted by Nguendo-Yongsi *et al*. (2004) identified 1242 isolates of *Enterobacteriaceae* family from a variety of drinking water, which *Shigella* species had 0.24% incidence, which was lower than the present study. The finding of this study confirmed the prevalence of *Shigella* species in schools' water and indicates, that it might be due to poor sanitation, mixing of sewage effluents with drinking water and due to the fecal contamination of drinking water. According to study conducted by Saima *et al*. (2018) in Pakistan, 22% of water samples were contaminated with *Shigella,* which is lower than the current study. In Pakistan 74% from tape, storage tanks and tube well were identified contaminated with *E. coli*, followed by *Salmonella* spp. (54%) and *Shigella* spp. (40%) (Mian *et al*., 2020). These findings are in line in the term of the prevalence of *Salmonella*, but the prevalence of *Shigella* was lower and the prevalence of *E. coli* was higher than the findings of current study, and this difference may be due to geographical location and different sanitation status. Several Researchers have been documented several outbreaks of Shigellosis, Salmonellosis and Colibacilosis were and reported the prevalence of 60% for *E. coli* which is high, and 12% *Salmonella*, which is lower than current study (Rather *et al*., 2013). The difference may be due to the fact that the researchers in their study had also taken samples from stream and lake, mixture of sewage water with fresh water and due to the contamination of fresh water with fecal material. These findings were in contrast with the study reported by Aboh *et al*. (2015) in Nigeria, where *Shigella* and *Salmonella* were not detected in any of the well water samples.

The results of present study are also in consensus with the results of Adabara *et al*. (2011) in the term of *E. coli*, but is different in the term of *Shigella* (6.7%) and *Salmonella* (8.3%). According to Momtaz *et al*. (2013), the prevalence of *E. coli* and *Salmonella* were 7.58% and 0.89%, respectively. The prevalence is much lower than that reported in present study. This significant difference may be due to the source of the samples.

The collected water sample were highly contaminated in government and private schools of Jalalabad city. Moreover, the drinking water of both wells and tanks were highly contaminated with *E. coli* followed by *Shigella* and *Salmonella*. Furthermore, the prevalence of studied pathogens were associated with deepness of well, distance of well from toiled, type of school authority and tanks of water. Thus, the owners of the schools should pay attention to the control of *Salmonella, Shigella* and *E. coli* by considering the associated factors studied in present study and all relevant Departments should follow WHO standards of drinking water.

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Conflict of Interest

The authors declare that they do not have conflict of interests.

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