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Evaluation of microbial flora from Anbar and Kernel Sher Kali stream water, District Swabi, Khyber Pakhtunkhwa

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Abstract

The current study was conducted for microbial analysis, viable cell count and antibiotics sensitivity test of bacterial isolates from Anbar and Kernel Sher Kali streams water. Two samples were collected from two different areas of Anbar and Kernel Sher Kali streams water. These samples were screened for bacteriological and fungal study and antibiotics susceptibility testing for isolates. The water samples were analyzed for bacteriological study by Multiple Fermentation Tubes Methods and PDA media growth for fungal spores. The isolated bacteria were confirmed on the basis of indole test, citrate test, catalase test and urease test.The result of the study showed that *Escherichia Coli, Klebsiella oxytoca, Klebsiella pneumonia, Candida* and*pencillium chrysogenum* were present inboth samples. Most probable numberfor Anbar stream water sample was 15 per 100ml with 95% confidence low rate was 4.5 and high rate was 42 while MPN for kernel Sher kali stream water sample was 27 with 95% confidence low rate was 8.7 and high confidence rate was 94.According to Antibiotic susceptibility profile *E. coli* and *Klebsiella oxytoca* from Anbar sample showed resistance to Amoxicillin, Ampicillin, Penicillin G, teicoplanin whereas *Klebsiella oxytoca*also found resistant to Fosfamycin. Bacterial strains i.e. *Klebsiella pneumonia* and *E.coli* isolated from water stream of Kernel Sher Kali were found resistant to Ampicillin, Penicillin G, teicoplanin while. *Klebsiella pneumonia* was found resistant to Amoxicillin. CFU for the Anbar stream water were ranging from 8.8×10^3 to 4.2×10^5 and for the Kernel Sher Kali stream water were ranging from 1.1×10^4 to 3.6×10^5. Both samples contain coliform contamination and does not meet the WHO standard and unfit to use for swimming or house hold activities.

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Introduction

Water is the ultimate demand of human life, for the nutrients and entertainment purposes. Without water there is no life which continues to exist on the earth (Cabelli, 2003).Water is a basic demand for human life day to day action such as drinking, cooking, washing, aesthetics, bathing, swimming etc. Principal water resources in Pakistan depends on the flows of the Indus River and its branches, Jhelum, Chenab, Ravi, Beas and Sutlej; these are also the fundamental veins of Pakistan water bodies (Kahlown and Majeed, 2003).

Recreational water includes bathing and unprocessed water present in nature, stream and seas water. Natural or unrefined water is the capital source of swimming pool and stream water (Fair and Okun, 2001). In semi urban areas of Islamabad, high density of bacteria present in all stream water samples, in which the most notable is *E.coli* and *Coliforms*. Many gutter lines, municipal wastes and wild life are the principal contamination sources of Islamabad streams water. These streams used for swimming in most areas, which are the main reason of children illness in pre monsoon, monsoon and post monsoon (Jadoon *et al.*,2012).

The water standard is generally analyzed by detecting pathogens, predominantly faecal coliform bacteria, up to some amount of fungal spores and physio-chemical properties also included in the standard of water (Bezuidenhout *et al.*, 2002). Water serves as a medium for the imparting of pathogenic microorganisms, if not appropriately manage, leading to waterborne diseases and as a mean for the proliferation and dispersal of human related pathogens (Mulamattathil, *et al.*, 2004).

Stream adulterate by undeviating human intervention and by extrinsic order e.g., soil, dust, rain, discharges of untreated domestic sewage water and poor drainage system, storm water, agricultural runoff and homes polluted water releases into streams, waste water discharges in to fresh water is the medium origin of microorganisms including pathogens and coliforms bacteria (Cabral. 2010).

The inappropriate management of stream and pool water may cause complication in availability and quality of water, due to this water have a direct effect on human health, cause diseases such as dysentery, hepatitis, cholera, polio, diarrhea, typhoid trachoma, ear infection, eczema, skin rashes and colour change of skin (Kinge *et al.*, 2012). More than 1.4 million deaths of children take place due to systematic, gastro-intestinal and diarrhea diseases (Ramírez-Castillo *et al.*, 2015), while according to the WHO data children in Developing Countries mostly affected by diarrhea, which globally accounts for more than 1.5 million deaths each year. Animal's feaces discharged into streams which are the main cause of *coliforms, E. coli. Giardia protist, V. cholera*caused by poor sanitation and discharges of untreated domestic sewage water (WHO (2004).

Coliforms are wide class of bacteria present in our environment, including the feces of man and other warm-blooded animals. These are rod shaped, gram negative, non-spore forming and motile or non-motile bacteria. Coliform bacteria ferment lactose with vapor formation within 48 h at 35°C. Coliform bacteria are facultative anaerobic, grow as red colour colonies with metallic sheen within 24-48 h at 37 °C in a medium contain lactose and galactosidase reaction (AWWA 2005).

Distract Swabi peoples are middle class village people they fulfill their water requirements through hand pumps and wells present in their homes but mostly peoples used streams water for their daily activities and swimming purposes, which caused many diseases in them. Therefore this study was conducted for microbial analysis, viable cell count and antibiotics sensitivity test of bacterial isolates of Anbar and Kernel Sher Kali streams water.

Materials and methods

Sample Collection

Two samples were collected from stream water of Union Council Anbar and Kernel Sher, District Swabi

from in fall season of 2017 and were transferred to Department of Microbiology, stored aseptically at 4°C till further microbiological analysis.

Multiple Tube Fermentation Technique

Test was carried out in 3 steps. Presumptive test: Nine (09) tubes for each sample processing in which three (03) media tubes contained double strength Lauryl Trytose Broth were inoculated with 10ml water sample, three (03) tubes contained single strength media inoculated with 1ml water sample, and the remaining three (03) tubes contained single strength media were inoculated with 0.1mlwater sample, after incubation checked inoculated tubes for coliform detection.

Confirmatory test: All the fermentation tubes in which gas production were seen, was considered positive and further processed by Eosin Methylene Blue Agar medium plate. After incubation checked plates for the round metallic green sheen colour colonies.

Completed test: MacConkey broth was prepared in sterilized tubes. Separated colonies picked from cultured EMB agar plates and inoculated in a MacConkey broth tubes, after incubation period inoculated tubes check for acid and gas production (Akeju *et al*., 2015).

Gram staining

Gram staining was used for the identification of morphological characteristics of isolated bacteria. Isolated strains of bacteria were biochemically identified using Indole test, citrate test, catalase test and urease test according to standard protocols.(Holt, 1994).

Fungal analysis

PDA (Potato Dextrose Agar) media was used for fungal growth. (Nizamydeen *et al* 2014).

Antibiotic susceptibility test (AST)

AST of the isolated microorganisms to various antibiotics was done according to CLSI guidelines. (CLSI guidelines., 2015).

Colony forming unit (CFU)

Spread plate method was used for the calculation of CFU.(Agbagwa., 2012).

Results

The coliform contamination and fungal growth were seen in both stream water samples. The Sample with positive growth for coliform was confirmed on the basis of morphology and biochemical identification while fungal detection was confirmed on the basis of PDA media growth. The *Escherichia coli, Klebsiella oxytoca, Klebsiella pneumonia, Candida albac* and *Pencillium chrysogenum* were found in samples (Table 1).

Table 1. Detection of bacterial and fungal species in Stream water samples.

Sample No	Location	Bacterial detection	Fungal detection
1	Anbar	*Escherichia Coli* *Klebsiella oxytoca*	*Candida albacans*
2	Kernel sher kali	*Escherichia Coli* *Klebsiella pneumonia*	*Penicillium chrysogenum*

Most probable number for Anbar stream water was 15 per 100ml with 95% confidence low rate was 4.5 and high rate was 42 while MPN for Kernel Sher Kali stream water was 27 with 95% confidence low rate was 8.7 and high confidence rate was 94 (Table 2). According to National Associating of Testing Authorities CFU criteria for swimming and domestic purpose water is less than 100cfu /1ml of sample. CFU in this study recorded for the Anbar stream water were ranging from 8.8×10^3 to 4.2×10^5 and for the Kernel Sher Kali stream water were ranging from 1.1×10^4 to 3.6×10^5 (Table 3).

Table 2. MPN of Anbar (1) and Kernel Sher Kali (2) streams water samples.

Sample No.	Water Quantity (ml)	Total No oftubes	Positive No of tubes	MPN per 100 ml	confidence limits 95%	
					Low	High
1	10	3	1			
	1.0	3	2	15	4.5	42
	0.1	3	1			
2	10	3	2			
	1.0	3	1	27	8.7	94
	0.1	3	2			

According to Antibiotic susceptibility profile *E.coli* and *Klebsiella oxytoca*from Anbar sample showed resistance to Amoxicillin, Ampicillin, Penicillin G, teicoplanin whereas *Klebsiella oxytoca*also found resistant to Fosfamycin. Bacterial strains i.e.*Klebsiella pneumonia* and *E. coli* isolated from water stream of Kernel Sher Kali were found resistant to Ampicillin, Penicillin G, teicoplanin while. *Klebsiella pneumonia* was found resistant to Amoxicillin (Table 4 and Table 5).

Table 3. CFUof Anbar (1) and Kernel Sher Kali (2) streams water samples.

S.NO	Sample	Dilution Factor	CFU
1	Anbar Stream water	10^{-2}	8.8×10^{3}
		10^{-3}	6.7×10^{4}
		10^{-4}	4.2×10^{5}
2	Kernel Sher kali stream water	10^{-2}	1.1×10^{4}
		10^{-3}	6.4×10^{4}
		10^{-4}	3.6×10^{5}

Discussion

The microbial analysis of both stream water samples showed that the water was highly contaminated with coliform. Three bacterial strains *Escherichia Coli, Klebsiella oxytoca, Klebsiella pneumonia* and two fungal strains *Candida, Pencillium chrysogenum* isolated from both area stream water samples. A similar study was carried out in Rawal Lake Pakistan; the results showed that *E. coli, fecal coliform* and *total coliform* in the samples proving it unfit for human use (Hassan and Hanif. 2014).

Table 4. Antibiotics susceptibility pattern of *E.coli* and *klebsiellaoxytoca*isolated for Anbar.

E. coli		*Klebsiellaoxytoca*	
Antibiotics	Interpretation	Antibiotics	Interpretation
Amoxycillin	R	Amoxycillin	R
Amikacin	S	Amikacin	I
Ampicillin	R	Ampicillin	R
Ciprofloxacin	I	Ciprofloxacin	S
Ceftriaxone	S	Ceftriaxone	I
Fosfomycin	S	Fosfomycin	R
Levofloxacin	S	Levofloxacin	S
Penicillin G	R	Penicillin G	R
Imipenem	S	Imipenem	S
Tazobactam	S	Tazobactam	I
Ofloxacin	S	Ofloxacin	I
Norfloxacin	S	Norfloxacin	S
Teicoplanin	R	Teicoplanin	R

In Hawaiian coastal streams study reported that *Salmonella, Campylobacter, Staphylococcus, Vibrio vulnificus,* and *V. parahaemolyticus, enterococci, Escherichia coli* were widespread in stream water, on the basis of these indicator microorganisms stream water not suitable for human activities (Viau, 2011).In this study all the isolates were MDR, as compared with the previous study of Cauvery River (Skariyachan, 2015). The previous study can be compared with the current study in which *E. coli* and *coliform bacteria* were isolated from the stream water bodies' samples.

Table 5. Antibiotics susceptibility pattern of *E. coli* and *Klebsiella pneumonia* isolated from Kernal Sher Kallay.

E.coli		*Klebsiella pneumonia*	
Antibiotics	Interpretation	Antibiotics	Interpretation
Amoxycillin	S	Amoxycillin	R
Amikacin	S	Amikacin	S
Ampicillin	R	Ampicillin	R
Ciprofloxacin	S	Ciprofloxacin	S
Ceftriaxone	S	Ceftriaxone	S
Fosfomycin	S	Fosfomycin	I
Levofloxacin	S	Ofloxacin	S
Penicillin G	R	Penicillin G	R
Imipenem	S	Imipenem	S
Tazobactam	S	Tazobactam	S
Ofloxacin	S	Ofloxacin	I
Norfloxacin	S	Norfloxacin	S
Teicoplanin	R	Teicoplanin	R

The CFU in this study was recorded for the Anbar and Kernal Sher khan stream water samples were ranging from 8.8×10^3 to $4.2\times10^5$and 1.1×10^4 to 3.6×10^5respectively, which is more than the CFU range recorded in the previous study (Ihuma, JO *et al*) While the CFU range studied in the current study was found less than CFU range recorded in the previous study (Maqbool *et al* 2016).

Microbial analysis of Okada town, stream water Edo state, Nigeria isolated microorganism were *Staphylococcus aureus, Salmonella species, Escherichia coli, Pseudomonas aerugionosa, Bacillus species* and *Flavobacterium*. MPN range from 7mpn/100ml to 14mpn/100ml (Sunday *et al* 2014). By comparing previous study with the current study it is indicated that MPN is less in the previous study.

Conclusion

Both samples were found contaminated with pathogenic species of bacteria and fungi. The isolated strains of bacteria were multi drug resistant, which are an alarming issue and can be the cause of infectious diseases in studied area.

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