



## ABSTRACT

This research work entitled “Assessment of Hand Hygiene Status of Some Students Using *E. coli* as a Major indicator (A Case Study, Federal Polytechnic, Mubi - Adamawa State)”. The main objectives of the study were to confirm coliform contaminants in the hands of the students of the department and to determine the sex with the highest rate of coliform contamination. The hands of

# ASSESSMENT OF HAND HYGIENE STATUS OF SOME STUDENTS USING *E. COLI* AS A MAJOR INDICATOR (A CASE STUDY, FEDERAL POLYTECHNIC, MUBI - ADAMAWA STATE)

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## Introduction

Faecal contamination is the major thing to look out for in the water and foods to be consumed by humans, this is simply because indication of faecal contamination shows that there is high chances that people can be infected with many intestinal diseases caused by enteropathogenic organisms especially *E. coli*, *Salmonella typhi* and polio virus. *Escherichia coli* occur in diverse forms in nature ranging from commensal strains to those pathogenic to humans or animal host.

On the basis of geometric information the species has been divided into six major different phylogenetic groups denoted as A, B1, B2, C, D, and E. (Touchon *et al.*, 2009). These subgroups encompass saprophytic (A) and pathogenic (in particular B2 types and are often considered to be the result of long (>1 year) evaluation time



although the physiology, generates and biochemistry of *E. coli* have been intensively studied but it is not known in details how the bacterium behaves in the natural habitats such habitats have been divided in primary that is animal human host associated (Macfarland and Nacarlane, 1997) and secondary that is open or non-host associated habitats (Savageall, 1983). The versatile behaviour exhibited by *E. coli* in these habitats is reflected in the immense diversity within the species (Berg thorsson and pathogenic forms of *E. coli*) are known to possess genomes that may differ by up to 20 % (Ochman and Jones, 2000). The phenotypes of the different *E. coli* forms are ensuring genomic islands in *E. coli* that define the different behavioural types are already an underpinning factor (Dobrindt et al., 2004; Touchon et al., 2009). The commensal form of *E. coli* exemplified by strain MG1655 (Touchon et al., 2009) is traditionally

thirty (30) students comprising of fifteen (15) males and fifteen (15) females were well washed using sterile water but without soap. The waste water obtained from sterile hand wash of 30 students were collected in sterile beakers and labeled accordingly. The samples were analyzed using 3 test tubes method of MPN and the result was interpreted using the three (3) test tubes MPN table. The male samples had the highest MPN with 150 CFU/mL, while females had 120 CFU/mL. Results obtained revealed that all samples were positive for coliform bacteria in presumptive test but only 12(80.0 %) male students have their hands contaminated with coliform bacteria while, 8(53.3 %) female students sampled were positive for confirmatory coliform test. The results for the completed test showed that 6(40.00 %) of the male samples yielded colonies of green metallic sheen which confirmed *E. coli* on EMBA and Microscopically appeared as Gram negative facultatively anaerobic bacteria, while 3(20.0 %) was recorded as being the lowest rate of *E. coli* contamination in the female students out of the fifteen (15) samples which also had the green metallic sheen.

**Keywords:** Gram negative, Contamination, *E. coli*



considered as harmless bacterium that lives in the intestinal system of animals and assist its host in the breakdown of particular carbon compounds. On the other hand, the pathogenic forms of *E. coli* such as the verotoxigenic *E. coli* (VTEC) (Taylor, 2008). Enterohaemorrhagic (EHEC) are pathogenic/ intractintestinal isolates that are harmful to their host. The well-known *E. coli* 0157: H7 is an example of a harmful VTEC, which has already caused high mortality worldwide causing bloody diarrhoea, with syndrome. More than 150 serotypes of verotoxin outbreaks are related to serotypes 0157, *E. coli* 0157: H7 is dangerous because of its resistance to low pH(-2.5), which allows passage through the stomach, its low infective dose (as few as 10 cells) and its high pathogenicity dose (Tilden *et al.*, 1996) and its low infective dose (as few as 10 cells) and its high tissue invasion, may ever cause death (it can be transferable to non-pathogenic *E. coli* strains, allowing these to enhance their virulence (Herold *et al.*, 2004).

In the light of the current question concerning the genomic make up, origin and environmental circulation into particular pathogenic *E. coli* this reviews will examine our current knowledge of the fate of different *E. coli* forms in open environment, and how it is carried in the hands people while contaminating other equipment and its distribution via shaking of hands.

The transfer of the different *E. coli* strains can be through contaminated nares and non-proper washing of hands which has been identified as the means transfer of the pathogenic strains, so proper hand washing have been encouraged by W.H.O to further improve on personal hygiene among others.

### **Classification of *E. coli* Pathotypes**

*E. coli* has been classified into six (6) pathotypes based on the virulence factors present and the symptoms they present in their host of diarrheogenic types namely: Enteropathogenic *Escherichia coli* (EPEC) cause diarrhoea in children and animals, Enterohemorrhagic *E. coli* EHEC which is responsible for hemorrhagic colitis and Hemolytic-Uremic syndrome, Enterotoxigenic *E. coli* (ETEC) Which causes traveler diarrhoea, Enteroaggregative *E. coli* (EAEC), which causes persistent diarrhoea in humans, Diffusely Adherent *E. coli* (DAEC) a subclass of



enteroaggregative *E. coli*, which causes diarrhoea in children, enteroinvasive *E. coli* (EIEC) which causes watery diarrhoea and dysentery, Uropathogenic *E. coli*

*E. coli* (DEC), promotes attaching-effacing lesions in eukaryotic cells. These lesions are mediated by intimin, an outer membrane adhesive protein encoded by the EAE (*E. coli* attaching effacing) gene is currently subdivided into typical and atypical subgroups. While typical EPEC carry the EPEC adherence factor plasmid (PEAF) that encodes the bundle forming pilus (BFP) and a complex regulator of various virulence gene (*per*) (Keawcharoen, 2004). Atypical EPEC is devoid of PEAF (or does not express a functional BFP). Typical EPEC express the localized pattern of adherence (LA), which is characterized by compact bacterial clusters on Hela and HEP-2 cell (Keawcharoen, 2004). Conversely, atypical EPEC most often express the LA-like pattern (with loose bacterial clusters) or adherence patterns of other EDE pathotypes.

#### ***Entero aggregative E. coli (EAEC)***

Another EDC pathotype, is identified by the characteristic aggregative pattern of adherence (AA) in Hela/HEP2 cell; bacteria attach in aggregates to cells surfaces as well as around cell (Songsirm, 2006). EAEE colonizes the intestinal mucosa, forming a thick biofilm that favours prolonged colonization and induces malnutrition (Songserm, 2006) pathotype is heterogeneous regarding the presence of putative virulence gene and has recently been subgrouped into typical and atypical EAEC, which carry and lack AggR (a global regulator of EAEC virulence), respectively (Songserm, 2006).

We recently conducted a study at the Institution de Puericulture Pediatria Martago Gesteria in Rio de Janeiro, Brazil, on the etiology of diarrhea affecting children of low socioeconomic status (Girao *et al.*, 2008). In the study, all *E. coli* isolate were analyzed regarding their adherence patterns in Hela cells and the presence of specific of virulence genes of the DEC pathotypes, according to previously reported method (Amonsin, 2006). Among 481 children (<2 years old) with diarrhea who were examined. 16(3.3 %) carried *E. coli* strains that co-expressed LA and AA (LA/AA), a phenotype not found among strains of 99 control children without diarrhea at the same hospital. The LA/AA phenotype was



confirmed in individual colonies of each strain as well as in HEP-2 cell. In both cell lineages, prolonged assays for 6 hours shows that a mature biofilm that masked the LA phenotype had developed. Although LA/AA co-expression in some human *E. coli* has been previously reported by Bouzari *et al.*(2004).

Moreover, since the expression of LA and AA is used to classify fecal *E. coli* as typical EPEC and EAEC (Songserm, 2006), respectively, the classification of such strains within the DEC pathotypes is difficult. To determine their most appropriate classification, we further characterized the 16 LA/AA strains of our collection. Colony hybridization assays used to search for additional *E. coli* virulence genes (*bfPA*, *pera*, *E-hly*, *daac*, *cdt*, *cnf*, *hly*, *aggR*, *aggC*, *aggC*, *aap*, *shf*, *irp2*, *pet*, *pic*, *ast A*, *pap*, *afa*, *sfa*, *efa*, *paa*, *saa*, *enfA*) this shows that all strains carried *eae*, *bfpa* and *pera*, and 13 also carried the EAF sequence (a cryptic PEAf marker). Less commonly found genes were *paa*, *shf*, *irp2*, *astA* and *efa*, and the remaining genes were absent. BFP expression was confirmed in all strains by immunoblot, and positivity in the fluorescent actin staining assay demonstrated that they can produce attaching /effacing lesions. PCR analysis of 4 (a, b, y and s) of at least ten (10) recognized intimin subtypes showed that subtype was the most frequent serotyping identified at least 10 distinct serotypes among the 16 strains, which demonstrated that they do not make up a single clone (Kurten, 2004). Two serotypes (0119:H6 and 0142:H6) are commonly found among typical EPE (Amonsin, 2005) certain typical and atypical EPEC serotypes have been associated with distinct intimin Subtypes.

Likewise, our LA/AA strains of the same serotype carried the same intimin subtype recently detected LA/AA expression in 4 of 21 *eae*-positive *E. coli* strains isolated from monkeys with diarrhea.(Carvalho *et al.*,2006). All four (4) strains expressed BFP and lacked the EAF sequence; as in our study, I belong to serotype 0142: H6 and carried intimin a. *E. coli* classification within the DEC pathotypes has epidemiologic and clinical implications for managing diarrhea diseases. However, finding *E. coli* isolates that co-express LA/AA reiterates the difficulty of assigning bacteria to groups on the basis of their adherence phenotype or genotype (particularly when based on mobile genetics elements). Since our analysis with molecular methods showed that these strains carry



more characteristics of typical EPEC and lack the *AggR* regulon, we propose that they be classified as typical EPEC. Typical EPEC are recognized as pathogens whereas atypical EAEC are not (Songserm, 2006).

In new addition, the ability to simultaneously induce attaching effacing lesions and biofilm production may increase the potential of the strains to cause diarrhea and prolong bacterial residence in the intestines, thus worsening malnutrition in the patient.

### ***E. coli* as an Indicator Organism in Water**

The is one of the major water quality questions every user of an estuary wants to know when preparing, boating, fishing, shell fishing, or other pursuit is whether the water is safe? Depends in part on the presence or absence of pathogens-virus, bacteria, and protozoans that can cause disease. Increasingly, monitoring and regulatory emphasis are focused on the potential for pathogens that may lead to waterborne diseases. Pathogens can enter a water body via fecal contamination as a result of inadequately treated sewage, faulty or leaky septic system, run off from urban areas, boat and marine waste, combined sewer overflows, and waste from pets farm animals, and wildlife. Human illness can result from drinking or swimming in water that contains pathogens or from eating shellfish harvested from such water.

Direct testing for pathogens is very expensive and impractical, because pathogens are rarely found in water bodies. Instead, monitoring for pathogens uses “indication” species -50 called because their presence indicates that faecal contamination may have occurred. The four indicators most commonly used today by both volunteer and professional monitors-total coliforms, fecal coliforms, *E. coli*, and enterococci-are bacteria that are normally prevalent in the intestines and feces of warm-blooded animals, including wildlife, farm animals, pets, and humans.

The indicator is usually pathogenic. The factors that should be considered when establishing a volunteer monitoring program for bacteria indicators and the analytical methods most commonly used to test for them should be well established. Case studies provide further examples and illustrations.



### **The Bacterial Indicators**

In this section the four main indicators. The testing methods which easily detect bacteria are discussed but before any microbiological analysis is done there is need to know the density of the indicator organism understand the criteria that were used to select should have some direct relationship to them as indicators to be an ideal assessor of the degree of fecal pollution (Gerba, 2002).

### ***Escherichia coli and Enterococci***

Other commonly used indicator bacteria are *Escherichia coli*, a single species within the fecal coliforms group, and enterococci, another group of bacteria found primarily in the intestinal tract of warm-blooded animals. Enterococci are unrelated to the coliforms; instead, they are a subgroup of the fecal streptococci group.

The method approved by the S.U. Environmental protection agency (EPA) for enterococci testing requires the use of an expensive growth medium that contains a toxic ingredient. Volunteer programs interested in monitoring for enterococci bacteria could partner with a university laboratory to conduct these tests.

### ***Effectiveness of Bacterial Indicators***

Total coliforms, faecal coliforms, *E. coli*, and enterococci are easy to grow in the laboratory and all will be present in large number if recent faecal contamination has occurred. Unfortunately, one problem with the indicators is the question of source. All the indicators can come from animals and some can also come from soil. Another problem is that none of the indicators accurately reflect the potential for human health effects, though some do a better job than others. Because of these and other complications, microbiologists are still looking for better indicators. In 1986, EPA issued a revision to its bacteriological ambient water quality criteria recommendations to include *E. coli* and enterococci, as they provide better correlations with swimming associated gastrointestinal illness than faecal coliforms. As an indicator, *E. coli* has a major advantage over the faecal coliform because is more of fecal specific (*E. coli* occurs only in the faeces of warm-blood mammals).



### **Statement of the Problem**

Hand washing is a very important aspect of personal hygiene. The world Health Organization stated that 70 % of infectious diseases can be prevented if only hands are properly washed with detergents and water after using the toilet or after washing an infant or when hands are soiled (World Health Organisation, 2009). The inadequate washing of hands has led to diarrhoea and other oral-faecal diseases.

### **Significance of the Study**

This study seeks to establish the level of faecal contaminants, since the students are mostly exposed to different types of contamination agents and possible cross contamination factors as they relate continuously daily. The outcome of this study will show the extent of contamination of hands of students by coliform bacteria which is a major indicator of possible faecal contamination. This will also serve as a wakeup call to students to always practice proper routine hand washing in line the standard procedure to effectively solve the problems of ineffective hand washing which has left so many people infected by different germs through hand shaking, hugging e.t.c as a social practice by most students and individuals.

### **Aim of the Study**

To determine the level of coliform contamination of the hands of some students of the Department of Biomedical and Pharmaceutical Technology, Federal Polytechnic, Mubi

### **Specific Objectives of the study**

- To confirm coliform contaminants in the hands of the students of the department.
- To determine the sex with the highest rate of coliform contamination.

### **Materials and Methods**

Sterile open mouthed containers, sterile distilled water, test tubes, durham tubes, lactose broth, nutrient agar, petri dishes, Gram's staining





reagents, wire loop, MPN table (3 test tubes), Eosine methylene blue agar, microscope and glass slides.

### ***Collection of samples***

A total of thirty (30) samples were collected in all. Fifteen (15) samples of waste water from hand washings were collected each from both male and female students of the Department of Biomedical and Pharmaceutical Technology, Federal Polytechnic, Mubi. The hands were carefully but thoroughly washed into well closed sterile open mouthed container and was carefully closed and transferred to the Microbiology Laboratory of the same department for Most Probable Number (MPN) analysis.

### ***Preparation of culture media***

Lactose broth of single strength and double strength, Nutrient agar and Eosine Methylene Blue Agar powder were weighed accordingly and prepared based on manufactures instruction and were kept at 4 °C for further usage.

### ***MPN (Most Problem Number)***

#### ***Arrangement and sterilization of the MPN Set up***

After the arrangement of the test tubes containing durham tubes for three test tubes method of MPN with control in place, the concentrations of single strength, single strength and double strength. Five (5 ml) each of two (2) single strengths and double strengths were measured using sterile 50 ml measuring cylinder into the appropriately labeled test tubes. The test tubes and their content were carefully tilted to expel air from the durham tubes and allowing the lactose broth to flow into the durham tubes and at this point the set up were sterilized at 121°C for 15minutes.

#### ***Presumptive test for MPN***

After completion of the sterilization of the MPN set up and was allowed to cool to 45°C, the hand wash waste water was inoculated into lactose broth in their respective volumes as describe by Chikezie (2014). Ten (10 ml) sample of waste water was inoculated using sterile techniques into



the first single strength broth, and 1ml sample inoculated into the second single strength and lastly 0.1ml sample was inoculated into the double strength lactose broth. All the tubes were carefully clogged with cotton wool and were incubated at 37 °C for 24 hours. The number of tubes that produced gas which can be seen from cloudy appearance in the tube was noted for each concentration of broth and volume of sample inoculated. The numbers of positive tubes were compared to the standard MPN index table for three test tube method to get the presumed number of coliform forming unit per milliliter sample analyzed.

### ***Confirmatory test***

Samples in the test tubes showing positive results and that of negative result from the presumptive test were taken using sterile wire loop. The loopful cultures of the samples were inoculated in test tubes in which durham tubes were placed alongside lactose broth as described by Chikezie, (2014). The two test tubes were incubated at 37 °C for 24 hours for possible gas production. If the sample that was previously positive for gas production still turns positive then this a positive confirmed test for coliform bacteria in the sample.

### ***Completed test***

In the completed test, the test tube showing positive test result from the confirmatory test was then inoculated and maintained in nutrient agar slant. Then the maintained isolates were inoculated on to Eosin Methylene blue agar and incubated at 37 °C for 24 hours. The appearance of colonies of shining green metallic sheen is indicative of a positive completed test of coliforms present in the hand wash waste water.

## **Results**

### **The occurrence of Highest MPN index based on sex**

The hands of the male students in this study were found to be contaminated with coliforms more than the female students as can be seen in table 1. The male student had the highest MPN of 150 CFU/mL, followed by the female students with the highest MPN of 120 CFU/mL from the presumptive test result as correlated on the MPN table for three test tubes methods used in interpreting the presumptive coliform



count. Table 1 also indicates the samples for which colonies of shiny green metallic sheen was obtained and this was found to be highest for the males as well.

Table1: Occurrence of the highest MPN and positive completed test for coliforms based on sex

Sample I.D	Pos. tubes			MPN	in	Completed test	Sample I.D	Pos. tubes			MPN	in	Completed test
Male	Ss	Ss	Ds	CFU/mL			Female	Ss	Ss	Ds	CFU/mL		
	10ml	1ml	0.1ml					10ml	1ml	0.1ml			
M1	3	2	1	150		GMS	F1	0	0	1	3.0		
M2	1	1	0	7.3			F2	2	0	1	14		GMS
M3	2	0	0	9.1			F3	0	0	1	3.0		
M4	3	1	2	120		GMS	F4	2	0	0	9.1		
M5	2	0	1	14			F5	0	0	1	3.0		GMS
M6	3	1	2	120		GMS	F6	3	1	2	120		
M7	2	2	0	21			F7	2	0	0	9.1		
M8	2	0	0	9.1			F8	0	1	0	3.0		
M9	3	1	2	120		GMS	F9	3	1	2	120		GMS
M10	3	1	2	120		GMS	F10	3	1	2	120		
M11	2	0	0	9.1			F11	2	0	1	14		
M12	2	2	1	28			F12	3	1	2	120		
M13	0	0	1	3.0			F13	2	2	0	21		
M14	2	2	1	28		GMS	F14	2	0	0	9.1		
M15	3	1	2	120			F15	0	0	1	3.0		

KEY: GMS= Green Metallic Sheen

### Confirmed Coliform Contamination of Hands of Students

The hands of total number of thirty students comprising 15 males and 15 females revealed that all samples were positive for presumptive test but only 12(80%) male students have their hands contaminated with coliform bacteria as shown in table one from the confirmatory test while, 8(53.3%) female students sampled were positive for confirmatory test as shown in table 2.



Table 2: Occurrence of rate of contamination of the hands of students based on sex drawn from MPN analysis.

Sex	No. of sample collected	% No. of samples positive for presumptive test	% No. of samples positive for confirmatory test	% No. of samples positive for completed test	Gram stain results
M	15	15(100.00)	12(80.00)	6(40.00)	Gram (- Ve) <i>E. coli</i> present
F	15	15(100.00)	8(53.30)	3(20.00)	Gram (- Ve) <i>E. coli</i> present

### Discussion

From the results obtained it is clear that the hands of most the students of the Department of Biomedical and Pharmaceutical Technology sampled in this study had their hands contaminated with the male students taking the lead of the most contaminated hands by coliform bacteria as the highest MPN of 150 CFU/ml and four (4) of the sample showed 120 CFU/ml, while the least had 28 CFU/mL for 6(40 %) of students out of a total of 15 of the male samples. All the six (6) male samples were confirmed in the completed test to be *E. coli* from the appearance of the colonies with the characteristic shiny green metallic sheen. Only 3(20 %) of female students had MPN of 120 CFU/ml out of the fifteen (15) of the students sampled and analyzed. In any of the of the cases the risk still remain that the number of coliform from the result is alarming for the male and females in line the a research that proves that some enteric bacteria are able to cause infections at a dose as low as 10 cell (Edberg *et al.*, 2000; Leclerc *et al.*, 2002).

The findings from this work has clearly indicate that there is possible chances of cross contamination among the students of the department with greater risk among the male students as they appear to take the lead in the rate of contamination by coliforms. It is important to realize that the *E. coli* found in this study is only an indicator suggest of direct possible faecal contamination or cross contamination from social



interactions among student and even contaminations from door handles which is another major proven means through which hands can be contaminated by faecal coliforms which is in agreement with the findings of Williams. (1997) that direct person-to-person infection of *E. coli* O157:H7 occurs particularly among children and their caregivers, such as in day care facilities and also within families.

The *E. coli* contaminant confirmed in this study is an indicator organism that is suggestive that other contaminating faecal pathogens such as *Salmonella Typhi*, *Vibrio cholerae* and other enteroviruses could also be present and this is in support of directives issued in 1986 by EPA on a revision to its bacteriological ambient water quality criteria recommendations to include *E. coli* and enterococci, as they provide better correlations with swimming-associated gastrointestinal illness than faecal coliforms.

### **Conclusion**

Considering the findings in this work, contamination of male students hand by *E. coli* is at its highest of 150 CFU/mL Which is an evident proof of need for increased hand washing with soap and water, detergent and water or ashes and water among students of the department so as to avoid being infected with faecal-oral diseases and other gastro-intestinal diseases which could in turn spread amongst students of the institution.

### **Recommendations**

Based on the outcome of this work the following recommendations were made;

- The international washing day set aside by WHO should be strongly recognized to emphasize the importance of hand washing and its role in preventing infectious diseases.
- More studies should be done to cover the entire department to ascertain the rate of contamination of *E. coli*.
- Hand washing components should be placed at strategic locations in the schools and other public places for effective hand washing practices.

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