Correlation of faecal indicator bacteria in human stools and hospital wastewaters

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Correlation between faecal indicator bacteria in diarrheagenic stools and hospital wastewaters: implication on public health

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Abstract:

Background: Hospital wastewaters contain blends of inorganic, natural constituents and contaminants that carry significant health risk when released directly into the environment. The aim of this study is to investigate the correlation between faecal indicator bacteria in diarrheagenic stools and wastewaters generated in University of Medical Sciences Teaching Hospital complex, Akure, Nigeria.
Methodology: Quantification of faecal indicator bacteria was carried out on diarrheagenic faecal samples collected from 55 hospitalized patients and 68 wastewater samples from the medical laboratory science and laundry units of the hospital over of period of 12 weeks. Standard membrane filtration technique was performed using membrane intestinal enterococcus (m-ENT), membrane faecal coliform (m-FC), membrane lauryl sulphate (MLSA), eosin methylene blue (EMB) and Salmonella-Shigella (SS) agar plates, which were incubated at 37ºC for 24 hours (MLSA, EMB and SSA), 44ºC for 24 hours (m-FC); and 37ºC for 48 hours (m-ENT). Bacterial colonies on agar plates were counted and expressed as colony forming units (CFU) per 100ml of diarrheagenic stool and wastewater. Pearson's correlation analysis was used to determine the relationship between the level of faecal indicator bacteria in diarrheagenic stools and wastewaters at $p<0.05$ level of significance (and 95% confidence interval).
Results: The faecal coliform counts (log 10 CFU/100ml) ranged from 1.18 to 1.54 in diarrheagenic stools, 1.32 to1.64 in laboratory wastewater and 1.08 to 2.19 in laundry wastewater. *Escherichia coli* count (log 10 CFU/100ml) ranged from 1.08 to 1.40 in diarrheagenic stools, 1.20 to 1.86 in laboratory wastewater and 0.30 to 1.81 in laundry wastewater. Intestinal enterococci count (log 10 CFU/100ml) ranged from 0 to 0.30 in diarrheagenic stools, 0.78 to 0.90 in laboratory wastewaters and 0.48 to 1.11 in laundry wastewaters. Pearson's correlation co-efficient showed that all the faecal indicator bacteria count in diarrheagenic faecal samples exhibited positive correlation with those in laboratory wastewaters, but not with those from laundry wastewaters.
Conclusion: The findings suggest that diarrheagenic stools should be properly disinfected after the performance of laboratory tests to prevent transmission of potential pathogens, and wastewater generated from hospitals should be treated prior to discharge into the environment, to prevent possible infections in the community.

Keywords: Correlation, faecal indicator bacteria, public health, transmission, wastewater

Corrélation entre les bactéries indicatrices fécales dans les selles diarrhéiques et les eaux usées des hôpitaux: implication sur la santé publique

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Abstrait:

Contexte: Les eaux usées des hôpitaux contiennent des mélanges de constituants inorganiques et naturels et de contaminants qui présentent des risques importants pour la santé lorsqu'ils sont rejetés directement dans l'environnement. Le but de cette étude est d'étudier la corrélation entre les bactéries indicatrices fécales dans les selles diarrhéiques et les eaux usées générées dans le complexe de l'hôpital universitaire des sciences médicales, Akure, Nigeria.

Méthodologie: La quantification des bactéries fécales indicatrices a été réalisée sur des échantillons fécaux diarrhéiques prélevés sur 55 patients hospitalisés et 68 échantillons d'eaux usées du laboratoire médical scientifique et des unités de blanchisserie de l'hôpital sur une période de 12 semaines. La technique de filtration membranaire standard a été réalisée à l'aide de plaques de gélose à entérocoque intestinal membranaire (m-ENT), coliforme fécal membranaire (m-FC), laurylsulfate membranaire (MLSA), éosine bleu de méthylène (EMB) et gélose Salmonella-Shigella (SS), qui étaient incubé à 37°C pendant 24 heures (MLSA, EMB et SSA), 44°C pendant 24 heures (m-FC); et 37°C pendant 48 heures (m-ENT). Les colonies bactériennes sur des plaques de gélose ont été comptées et exprimées en unités formant colonies (CFU) pour 100 ml de selles diarrhéiques et d'eaux usées. L'analyse de corrélation de Pearson a été utilisée pour déterminer la relation entre le niveau de bactéries fécales indicatrices dans les selles diarrhéiques et les eaux usées à p <0,05 niveau de signification (et intervalle de confiance à 95%).

Résultats: Le nombre de coliformes fécaux (log 10 CFU/100ml) variait de 1,18 à 1,54 dans les selles diarrhéiques, de 1,32 à 1,64 dans les eaux usées de laboratoire et de 1,08 à 2,19 dans les eaux usées de lessive. Le nombre d'*Escherichia coli* (log 10 UFC/100ml) variait de 1,08 à 1,40 dans les selles diarrhéiques, de 1,20 à 1,86 dans les eaux usées de laboratoire et de 0,30 à 1,81 dans les eaux usées de lessive. Le nombre d'entérocoques intestinaux (log 10 UFC/100ml) variait de 0 à 0,30 dans les selles diarrhéiques, de 0,78 à 0,90 dans les eaux usées de laboratoire et de 0,48 à 1,11 dans les eaux usées de lessive. Le coefficient de corrélation de Pearson a montré que tous les comptages de bactéries fécales indicatrices dans les échantillons fécaux diarrhéiques présentaient une corrélation positive avec ceux des eaux usées de laboratoire, mais pas avec ceux des eaux usées de lessive.

Conclusion: Les résultats suggèrent que les selles diarrhéiques doivent être correctement désinfectées après la réalisation des tests de laboratoire pour éviter la transmission d'agents pathogènes potentiels, et que les eaux usées générées par les hôpitaux doivent être traitées avant d'être rejetées dans l'environnement, afin de prévenir d'éventuelles infections dans la communauté.

Mots clés: corrélation, bactéries indicatrices fécales, santé publique, transmission, eaux usées

Introduction:

Hospice discharges remain a specific instance of anthropogenic contaminants. The aqueous hospital wastes are intricate blends of inorganic and natural constituents which are frequently dislodged into the immediate vicinity (1). This assortment is the consequence of analytical research laboratory outcomes. Waste and drugs including active by-products from pharmaceutical exhibits and their matters, synthetics, sterilizing agents, specific cleaners, irradiated indicators, bacteria and their anti-microbial resistant genes are sometimes present in hospital wastewaters (1). These wastewaters spawn from all therapeutic and non-medical activities from the laboratory to laundry activities (2). They are also a catalogue of microorganisms, disinfectants and drug wastes (3).

Faecal indicator bacterial (FIB) species are commensal-like bacteria found in the alimentary canal of warm-blooded animals, including humans (4,5). FIB species are used to examine faecal contamination intensities and therefore the prospect of pathogens of faecal source in soils and water in equally humid and temperate systems (6). FIB species comprise *Escherichia coli*, *Salmonella* spp, *Enterococcus* spp, and the coliforms. Globally, these groups of microorganisms are acceptable as useful indicators of faecal contamination since they exhibit close relationship with health hazards associated with gastrointestinal symptoms (7). *Escherichia coli* is reflected to be a more explicit pointer of faecal pollution than faecal coliforms since the broader test for faecal coliforms also detects thermotolerant non-faecal coliform bacteria (8). Enterococci are currently the lone FIB endorsed by the United States Environmental Protection Agency (9) for salty and marine waters, since they compare superiorly with human wellbeing effects than further FIB such as faecal coliforms or *E. coli* (10,11).

Most bacteria consortia of gastro-intestinal infections such as *Shigella*, *Campy-lobacter*, *Salmonella* and *Escherichia coli* are pathogenic in nature (12), one of which *Shigella* plays a central role in the occurrence of inflammatory diarrhea (13). Hospital-acquired gastroenteritis is an ordinary snag in hospitalized patients but its basis and implications are often taken for granted. Diarrhea illnesses among these patients may be associated with considerable morbidity or mortality (14,15,16). Hospital - acquired gastroenteritis constitutes a heightened incidence of diarrhea

in hospitalized patients that was not experienced prior to admission but commences after more than three days of hospital duration stay (17,18,19). Medically, this description is constructive since there is a possibility of community-acquired viral, bacterial or parasitic gastroenteritis budding later than the 3-day duration of hospital stay (17), although, in hospitalized patients, administration of anti-microbials and other medical approaches may cause diarrhea by unsettling the host-intestinal microbiome association (18,20,21,22).

Hospital wastewater may contain undesirable latent pathogens including antibiotic-resistant bacteria, and viruses (23,24). These innocuous agents, which may linger in wastewater treatment plants can aggravate contamination of the normal milieu by instigating natural bio-disproportion (25). The effluents from hospice wastewater treatment plants (WWTPs) are often discharged into surface waters (e.g., rivers, streams, lakes). Potential hazards as a result of these effluents in aquatic environments depend largely on the composition of the effluents, degree of treatment of the effluents, absorption of composites in wastewater, and water current rate of the receiving-river or stream (26). This contamination may lead to multiple environmentally-related risks such as sewage pollution of rivers, streams and other surrounding waters (27,28).

The World Health Organization (29) stated that about 85% of hospice wastes in the United States are harmless, 10% infective and 5% non-infective (but harmful), and infectivity outcomes may differ due to factors related to environmental setup (28). In the absence of hospital WWTPs, pathogenic bacteria may be discharged directly into aquatic reservoirs. The release of untreated hospice effluents directly into the environment may increase the volume of biological materials and critical nutrients that can impact variations in the quality of the receiving environment (30). In addition, hospital wastewater effluents are not odorless and microbial decomposition processes in the effluents may lead to air pollution and cause respiratory problems in humans residing in the surrounding environment and ecosystem (31).

The rationale for this study was to investigate the correlation between faecal indicator bacteria in diarrheagenic stools and wastewaters generated from the laboratory and laundry of the University of Medical Sciences Teaching Hospital Complex, Akure, Nigeria. This is to gain an improved comprehension of the risk of onward transmission of potential pathogens in hospital settings as well as the impact of untreated or partially treated hospital wastewaters discharged into the surrounding milieu.

Materials and method:

Study area

The study setting, the University of Medical Sciences Teaching Hospital, Akure (UNIMEDTH) (coordinates 7.2421º N, 5.1957º E), is a state-owned teaching hospital located in Akure South Local Government Area of Ondo State, Nigeria (Fig 1).
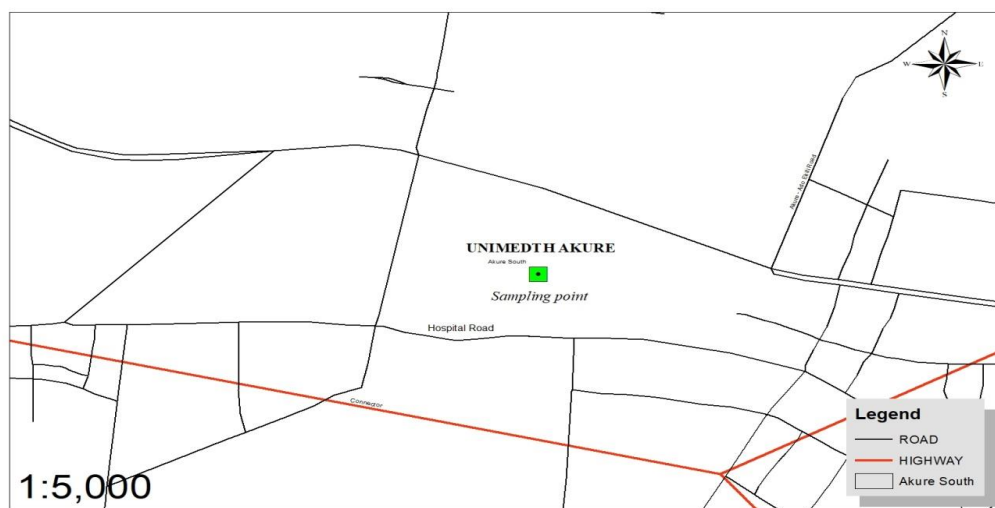

Fig. 1: Locality map showing University of Medical Sciences Teaching Hospital, Akure

Sample collection

Five millimeters of diarrheagenic faecal samples were collected from 55 hospitalized patients into sterile red-capped bottles and 500 ml of 68 wastewater samples were collected into sterile one litre plastic bottles from medical laboratory science and laundry units of the hospital over a period of 12 weeks. Samples were stored in ice packs at a temperature of 4ºC and analyzed within 1 hour of collection.

Enumeration of bacteria in diarrheagenic faecal stool and wastewater samples

Membrane filtration was performed according to the method of Maheux et al., (32), with slight modification. The selective media used; membrane intestinal enterococci (m-ENT), membrane faecal coliform (m-FC), membrane lauryl sulphate agar (MLSA), eosine methylene blue (EMB) and *Salmonella-Shigella* agar (SSA), were prepared according to the manufacturers' specification. The membrane filters (0.45μm, Delson Pascal Nig. Ltd) were positioned on the freshly prepared selective media. Agar plates were incubated at 37ºC for 24 hours (MLSA, EMB, SSA), 44ºC for 24 hours (m-FC) and 37ºC for 48 hours (m-ENT). Colonies (purple for faecal coliforms, metallic green for *E. coli*, dark brown for intestinal enterococci, black for *Salmonella* and colourless for *Shigella*) were counted using J 2 colony meter (Pec Medical, USA), calculated and expressed as colony-forming units (CFU) per 100ml of diarrheagenic stools and wastewaters (Fig 2).

Statistical analysis

Data obtained were converted to log10 and subjected to general descriptive statistics. Analysis of variance (ANOVA) and test of significance using Duncan's new multiple range test were undertaken using Statistical Package for the Social Sciences (SPSS) version 20.0. All data were analysed by Pearson's correlation at $p<0.05$ level of significance representing 95% confidence interval, to determine the relationship between the level of faecal indicator bacteria in diarrheagenic stools of hospitalized patients and wastewaters generated from the laboratory and laundry units.
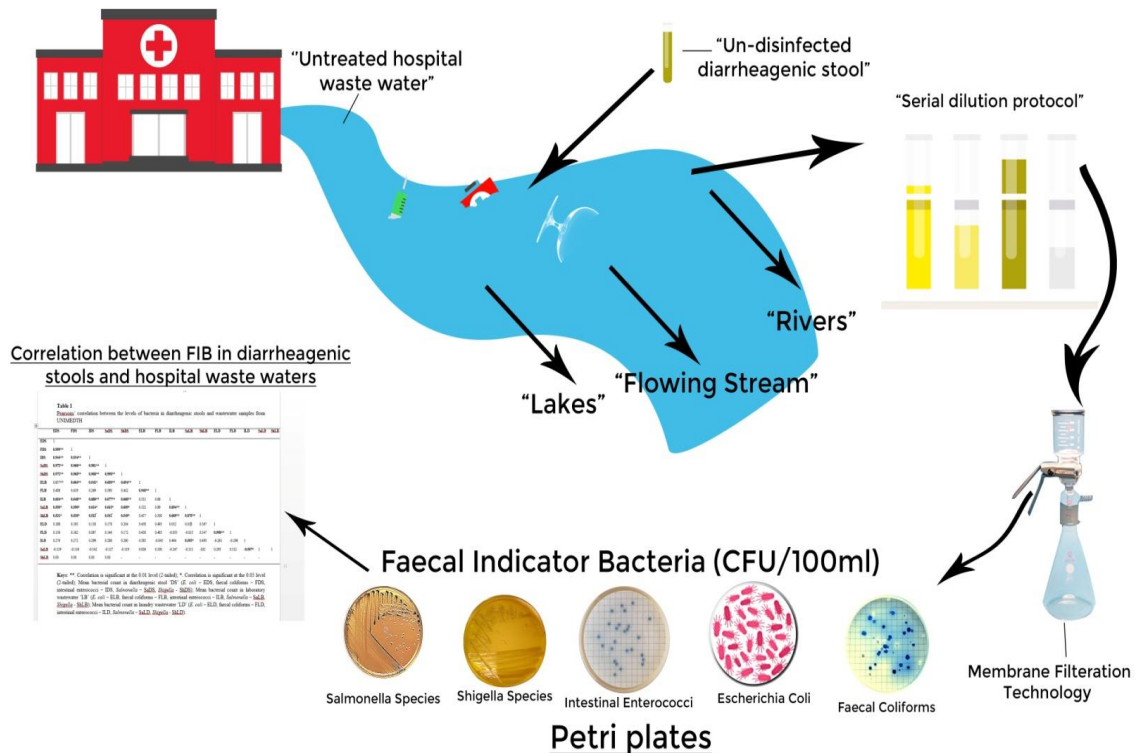

Fig. 2: A graphical illustration of the explanatory synopsis of the correlation of faecal marker bacteria in diarrheagenic stools and hospice effluents

Results:

Detection of bacteria in diarrheagenic faecal samples

Faecal coliforms had the highest mean count of 1.34 log10CFU/100ml while intestinal enterococci had the lowest mean count of 0.30 log10CFU/100ml in diarrheagenic stool. *Escherichia coli*, *Salmonella* spp, and *Shigella* spp had mean counts of 1.28 log10CFU/100ml, 1.04 log10CFU/100ml and 1.15 log10CFU/100 ml respectively (Fig 3).

Detection of bacteria in wastewater samples

Faecal coliforms had the highest mean count of 1.73 log10CFU/100ml and 1.72 log10 CFU/100ml in laboratory and laundry waste-water samples respectively while intestinal enterococci had the lowest mean count of 0.85 log10CFU/100ml and 0.95 log10CFU/100ml in laboratory and laundry wastewater samples respectively. The respective mean counts in laboratory and laundry wastewater samples for *E. coli* were 1.61 log10CFU/100ml and 1.69 log10CFU/100ml, *Salmonella* spp, 1.08 log10 CFU/100ml and 1.62 log10CFU/100ml, and *Shigella* spp 1.40 log10CFU/100ml and 1.53 log10CFU/100ml. The bacterial counts in the laundry wastewater samples were multiple than those in the laboratory wastewater samples (Fig 4).
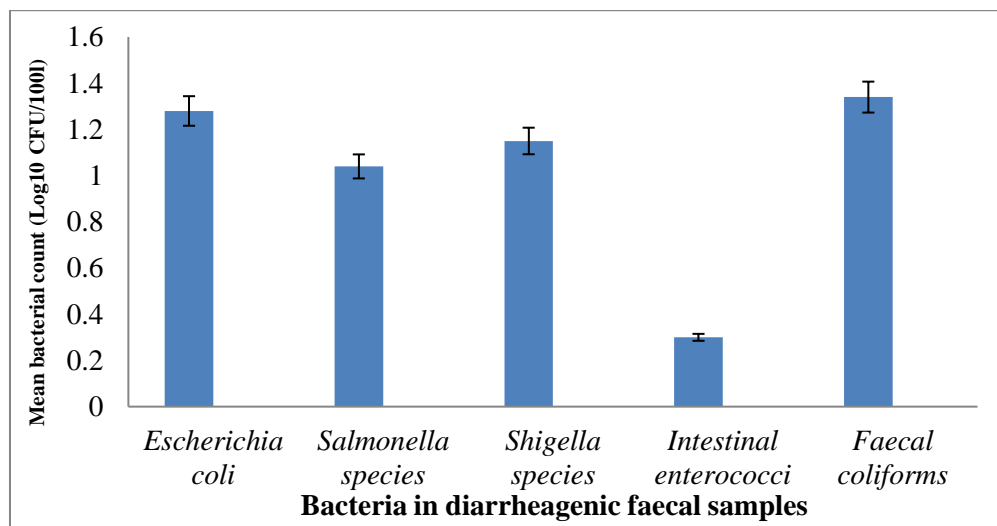

Fig. 3. Mean count of targeted bacterial indicators in diarrheagenic faecal samples
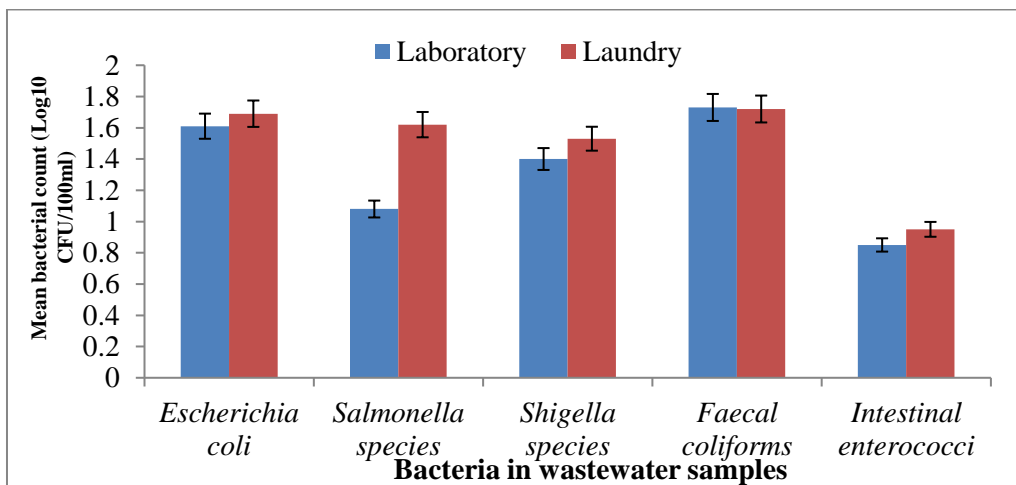

Fig. 4. Mean count of bacterial indicators in laboratory and laundry wastewater samples

Table 1: Pearson's correlation between bacteria counts in diarrheagenic stools and wastewater samples

	EDS	FDS	IDS	SaDS	ShDS	ELB	FLB	ILB	SaLB	ShLB	ELD	FLD	ILD	SaLD	ShLD
EDS	1														
FDS	0.999**	1													
IDS	0.944**	0.934**	1												
SaDS	0.972**	0.966**	0.981**	1											
ShDS	0.972**	0.968**	0.968**	0.998**	1										
ELB	0.657**	0.664**	0.542*	0.650**	0.694**	1									
FLB	0.408	0.419	0.269	0.390	0.442	0.940**	1								
ILB	0.654**	0.648**	0.680**	0.677**	0.668**	0.331	0.68	1							
SaLB	0.595*	0.590*	0.614*	0.615*	0.609*	0.322	0.89	0.894**	1						
ShLB	0.531*	0.530*	0.512	0.541	0.549*	0.457	0.300	0.669**	0.875**	1					
ELD	0.188	0.193	0.118	0.178	0.204	0.458	0.493	0.012	0.016	0.367	1				
FLD	0.156	0.162	0.087	0.146	0.172	0.438	0.485	-0.033	-0.015	0.347	0.998**	1			
ILD	0.276	0.272	0.299	0.288	0.280	0.383	-0.045	0.464	0.593*	0.493	-0.261	-0.298	1		
SaLD	-0.119	-0.116	-0.142	-0.127	-0.119	0.026	0.100	-0.247	-0.311	-202	0.293	0.312	-0.567*	1	1
ShLD	0.00	0.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed); Mean bacterial count in diarrheagenic stool 'DS' (*E. coli* – EDS, faecal coliforms – FDS, intestinal enterococci – IDS, *Salmonella* – SaDS, *Shigella* - ShDS); Mean bacterial count in laboratory wastewater 'LB' (*E. coli* – ELB, faecal coliforms – FLB, intestinal enterococci – ILB, *Salmonella* – SaLB, *Shigella* - ShLB); Mean bacterial count in laundry wastewater 'LD' (*E. coli* – ELD, faecal coliforms – FLD, intestinal enterococci – ILD, *Salmonella* – SaLD, *Shigella* - ShLD).

Relationship between levels of bacteria in diarrheagenic stools and wastewaters

The mean counts of *E. coli* in diarrheagenic stools showed positive correlation with the count of *E. coli* (r=0.657), intestinal enterococci (r=0.654), *Salmonella* (r=0.595), *Shigella* (r=0.531) in the laboratory wastewaters. In addition, the mean faecal coliform counts in diarrheagenic stools showed positive correlation with the count of *E. coli* (r=0.664), intestinal enterococci (r=0.648), *Salmonella* (r =0.590), *Shigella* (r=0.530) in the laboratory wastewaters.

Similarly, the mean bacterial counts of intestinal enterococci in diarrheagenic stools showed positive correlation with the count of *E. coli* (r=0.542), intestinal enterococci (r= 0.680), *Salmonella* (r=0.614), and *Shigella* (r= 0.512) in the laboratory wastewater. Also, the mean bacterial counts of *Salmonella* in diarrheagenic stools showed positive correlation with *E. coli* count (r=0.650), intestinal enterococci (r=0.677), *Salmonella* (r=0.615), and *Shigella* (r= 0.541) in the laboratory wastewaters.

The mean bacterial count of *Shigella* in diarrheagenic stools showed positive correlation with the count of *E. coli* (r=0.694), intestinal enterococci (r=0.668), *Salmonella* (r =0.609), and *Shigella* (r=0.549) in the laboratory wastewaters. There was no significant association between the levels of bacteria in diarrheagenic stools and the laundry wastewaters, with faecal coliforms (r=0.312), *Salmonella* (r=1) and *Shigella* (r=1) as shown in Table 1.

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Discussion:

In this study, the correlation between faecal indicator bacteria (FIB) in diarrheagenic stools and hospital wastewaters with the implications and impacts of FIB on human health was evaluated. The high faecal coliform counts in diarrheagenic stools is in agreement with the observations of Sinton et al., (33) where the authors inferred that faecal coliforms are abundant in human faeces. The intestinal enterococci count in diarrheagenic stools also agrees with the reports of Boehm and Soller (11), and Boehm and Sassoubre (34), which noted that enterococci are found in high concentrations in human faeces but at a lower concentration when compared to the level of *E. coli*. Studies have reported *E. coli* as a predominant inhabitant of the gastrointestinal tract of humans (4,5), and in human faeces, 90-100% of coliform bacteria isolated are *E. coli* (35). The large numbers of *E. coli* inhabiting the human gut and their absence in other environments backed their continual usage as the utmost subtle marker of faecal contamination (36).

Similarly, García-Aljaro et al., (37) reported that intestinal microbiome in healthy individuals serves as pointer to varying pathological conditions which cause diarrhea. This may account for the high *E. coli* count in diarrheagenic faecal samples in this study. The relatively high concentration of *Salmonella* species agrees with the findings of Jafari et al., (38) who observed high count of *Salmonella* spp from acute diarrhea patients at some hospitals in Tehran, and reported that *Salmonella* spp constitutes significant human health hazard in that it presents diarrhea as one of its major clinical symptoms in humans. In the same vein, the high count of *Shigella* spp in diarrheagenic stools in this study agrees with the observations of Majalan et al., (39) who detected high *Shigella* count among diarrhea patients admitted to certain hospitals in Tehran. Moghanloo et al., (40) also detected *Salmonella* and *Shigella* species in stools of patients with diarrhea in Kashan City, Iran.

The high faecal coliform count observed in laboratory and laundry wastewater samples agrees with the findings of Chukwu et al., (41) who detected high total faecal coliform count (TFCC) of 2.9×10^3 and 2.4×10^2 CFU/ml respectively from laboratory and laundry wastewater samples collected from Abia State University Teaching hospital, Aba, Nigeria. Our observation is also in agreement with Sadek et al., (42) who detected significant levels of faecal coliforms in hospital discharges, thus indicating the level of faecal pollution of the receiving environment. Studies have demonstrated that the discharge of raw wastewater from hospices may have undesirable consequences and a considerable impact on public health as a result of constituents such as pathogenic organisms also including antibiotic resistant organisms, innocuous chemicals, chemotherapeutic remains, pharmaceutical materials, and irradiated isotopes (43,44).

In our study, the low count of intestinal enterococci in laundry and laboratory wastewater samples agree with Moges et al., (45) who observed low levels of enterococci in wastewater samples from hospital environment in Northern Ethiopia. However, *E. coli* counts in laundry and laboratory wastewater samples were higher than those observed by Moges et al., (45). Other studies have established the presence of *E. coli* in habitats outside the gastrointestinal tract such as hospital wastewaters (45,46).

The correlation between counts of enteric bacteria in diarrheagenic stools with those in wastewaters generated in the laboratory in this study may not be unconnected with the fact that faecal marker bacteria are usually commensals of the alimentary tract of many warm-blooded animals especially humans, and are passed out in faeces in large quantities. These enteric bacteria are also present in considerable quantity in faecally-contaminated waters in the hospital environment (47). The positive correlation between *E. coli* in diarrheagenic stools and *E. coli*, intestinal enterococci, *Salmonella* and *Shigella* spp in laboratory wastewater could be a consequence of high rate of uninterrupted discharge of faecal samples from the laboratory after necessary diagnostic procedures into wastewater. This bears some semblance with the findings of Patra et al., (48) who showed positive significant correlation of *E. coli* in stool samples with presumptive *E. coli*, *Shigella*, and *Salmonella* spp in laboratory wastewaters.

The positive correlation of faecal coliforms in diarrheagenic stools with *E. coli*, intestinal enterococci, *Salmonella* and *Shigella* spp in laboratory wastewater samples may indicate the discharge of un-disinfected faeces into hospital effluents (49). This also buttress the observations of Patra et al., (48) who showed positive significant correlation of faecal coliforms in diarrheagenic stools with presumptive *E. coli*, *Shigella*, *Salmonella* spp in laboratory wastewaters. The significant correlation observed between intestinal enterococci in diarrheagenic stool samples with intestinal enterococci, *Salmonella* and *Shigella* spp in

laboratory wastewaters is comparable to the findings of Schriewer et al., (4) who observed significant correlation between intestinal enterococci from diarrheagenic faecal samples and *Salmonella* spp in laboratory wastewater samples. In contrast, a study conducted by Hatha et al., (50), observed no significant correlation between high levels of faecal coliforms in diarrheagenic stools and incidence of specific pathogens such as *Salmonella* and *Shigella* spp in laboratory wastewaters. The positive correlation observed between *Salmonella* spp in diarrheagenic stools and *E. coli*, intestinal enterococci, *Salmonella* and *Shigella* spp in laboratory wastewaters agrees with the findings of Efstratiou et al., (51), where the authors observed a strong direct correlation between indicator bacteria (enterococci and *E. coli*) in wastewater samples and *Salmonella* spp in stool samples but contrastingly, the study by Polo et al., (52) reported little or no correlation of indicator bacteria in hospital wastewater samples with *Salmonella* spp from stool samples.

In the study by McEgan et al., (53), weak direct correlation between natural markers (*E. coli* and coliforms) in hospital wastewaters and *Salmonella* levels in stool samples was reported, which may suggests that *E. coli* and coliforms are not absolute markers of the presence of pathogenic enteric bacteria such as *Salmonella* and *Shigella* spp in diarrheagenic stool samples, because there could also be contaminations by pathogens from non-faecal sources of the wastewaters in the hospital environment (54). The positive correlation reported between *Shigella* spp in diarrheagenic stools and levels of *E. coli*, intestinal enterococci, *Salmonella* and *Shigella* spp in hospital wastewater samples is comparable to the results of Ferguson et al., (55), who reported statistically significant correlations of bacterial pathogens such as *Salmonella* and *Shigella* spp in hospital wastewater with faecal indicators in stool samples.

Conclusion:

Our study demonstrates significant correlation between faecal indicator bacteria in diarrheagenic stools and laboratory wastewaters. The findings suggest that diarrheagenic stools should be disinfected after the performance of laboratory diagnostic tests in order to prevent onward transmission of potential pathogens in hospital settings. In addition, wastewater generated from hospitals must be fully treated prior to discharge into the environmental milieu in order to protect human health.

Conflict of interest:

Authors declare no conflict of interest.

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