THE USE OF PASSIVE SAMPLERS FOR THE DETECTION OF *E. COLI* IN WASTEWATER

GENERAL RESEARCH PROFILE

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ABSTRACT

Wastewater surveillance of antibiotic resistant bacteria and genes has been a useful epidemiology tool in monitoring antibiotic resistance within populations. These monitoring approaches are frequently reliant on composite samples from automatic samplers. These samplers can be expensive and require infrastructure modifications. Passive sampling could be a practical and affordable approach to wastewater surveillance in certain situations where automatic samplers cannot be implemented. However, their use in the detection of bacteria within wastewater is limited. The overall objective of this study was to assess the detection capacity of various passive materials for *E. coli* in wastewater. The casing used to house the passive materials was a torpedo shaped passive sampler. The passive materials placed in the sampler are gauze, cotton buds, and either a nylon or polyvinylidene fluoride (pvdf) membrane filter. The first aim of the study was to develop an effective protocol for the detachment of *E. coli* from different passive materials placed in wastewater. We determined that the use of peptone physiological salt and vortexing provided an effective approach for the detachment of *E. coli* from the materials. Also, in this study, passive samplers were placed once a week for a 24-h continuous sampling in effluent wastewater for 8 weeks. The highest quantity of *E. coli* was detected on gauze with mean concentrations of 4.9 log cfu / passive material, followed by bud (3.7 log cfu / passive material) while nylon and pvdf membranes had similar mean concentrations (2.3 log cfu/ passive material). Furthermore, buds (R^2 = 0.88) showed the strongest relationship when compared with the 24-h effluent composite samples collected from automated samplers, while gauze had the weakest relationship (R^2 = 0.48). The passive materials also showed that they could detect *E. coli* during exposure to varying concentration and time-duration. These results have demonstrated that the samplers can be used for passive sampling of bacteria within wastewater, however the type of passive material to be used is dependent on the research aims to be achieved.

LAYMAN SUMMARY

The monitoring of sewage that enters that enter wastewater systems can be a useful tool to monitor bacteria and genes that are resistant to treatment with antibiotics. This approach is usually achieved by using automated samplers which collect water samples at a regular time or volume which will reflect the average wastewater sample within that specific period. These automated samplers are usually expensive, require constant electricity to run and specialized individual to install it. In cases where this approach may not be applicable, using passive sampling might be a cost-effective approach. However, there is limited experience with the use of various passive materials for the detection of bacteria in wastewater.

The overall objective of this study was to evaluate if different passive materials can detect *E. coli* bacteria in wastewater. The passive sampler used in this study was a torpedo shaped sampling device. The sampler contained gauze, cotton bud, and either a nylon or polyvinylidene fluoride (pvdf) membrane filter. The first aim of this study was to develop a method of removing *E. coli* from the materials which can be replicable and standardized. The findings indicate that using peptone physiological salt and vortexing can be effective in the removal of *E. coli* from the passive materials.

Also, in the study, the passive samplers were placed in treated wastewater that would eventually be released into the environment. The samplers were placed in effluent for 24 hours within 8 weeks. The findings from this study show that the highest quantity of *E. coli* was detected on the gauze passive material with average concentrations of 4.9 log cfu / passive material, followed by the cotton bud (3.7 log cfu / passive material), while the membrane filters that were either made of nylon or polyvinylidene fluoride has similar quantity of *E. coli* detected (2.3 log cfu/ passive material). The passive samplers were also compared to the standard 24hr water samples gotten from the automatic samplers. The results showed that buds had a better relationship (R^2 = 0.88) when compared to concentrations from the automated sampler while gauze (R^2 = 0.48) had the weakest relationship among the passive materials. The study also showed that it was possible to use the passive samplers to detect *E. coli* in different concentrations and time duration. The findings from this study shows that passive sampler can be implemented for use within wastewater systems.

1.0 INTRODUCTION

Antibiotics are broadly applied for human, veterinary, and agricultural purposes. They can be used to treat infections in humans and animals and in some cases used as animal growth promoters (Bouki et al., 2013). The constant use and misuse of antibiotics may result in the development of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs). This can reduce the therapeutic potential against pathogens and pose a health risk to humans and animals (Kemper, 2008). Because antibiotics are not completely metabolized by the human or animal body, functional compounds can enter wastewater systems through human or animal waste (Kummerer, 2003). In addition, ARB and ARGs of enteric origin can also be released into the environment via fecal waste (Huijbers et al., 2020).

As a result, various factors including application of sewage sludge on farmland, and disposal of wastewater to surface waters can contribute to the presence of ARB and ARGs in different environmental components such as wastewater treatment plants (WWTPs) (Manaia et al., 2018; Michael et al., 2013), aquaculture (Cabello, 2006; Chen et al., 2018), surface and ground water (Leonard et al., 2015; O'Flaherty & Cummins, 2017) and soils (Cycoń et al., 2019; Jechalke et al., 2014). WWTPs are of particular interest because antibiotics, ARBs, and ARGs can pass through the treatment process to be discharged at low concentrations into the environment (Berendonk et al., 2015).

The sewage entering the WWTPs contains the pooled excreta produced from individuals in that area. Therefore, it is expected that WWTP influent mirrors at least in part, the circulation of ARB, resistance genes and associated mobile genetic elements within the population (Berendonk et al., 2015; Rizzo et al., 2013).Wastewater surveillance (WWS) which involves monitoring sewage from wastewater systems for ARBs and ARGs can be a cost-effective approach for antimicrobial resistance surveillance (Tiwari et al., 2022). It can also be used as an early prediction tool for future infection outbreaks. This could provide information that assists in policy making regarding public health interventions (Flach et al., 2021). This approach collects samples on a population level non-invasively and minimizes the ethical and privacy concerns involved with surveillance (Tiwari et al., 2022).

In recent years, a number of resistant bacteria has been detected in WWTPs, including extended spectrum beta-lactamase (ESBL) producing *E. coli* and carbapenemase-producing Enterobacterales (CPC). ESBL *E. coli* are resistant toward third and fourth generation beta-lactam antibiotics and only respond to carbapenem antibiotics. They have been found in wastewater (Blaak et al., 2015; Korzeniewska et al., 2013), and surface water (Blaak et al., 2021; Montezzi et al., 2015). The treatment options for infections due to ESBL E. coli are limited to carbapenems which are last-resort antibiotics and continued use creates further concerns of resistance. They have also been detected in hospital and municipal wastewater (Blaak et al., 2021; Montezzi et al., 2015). Consequently, this shows the possibility of using wastewater surveillance to monitor the distribution of these resistant bacteria in the population. For example, some studies have shown correlation between resistance rates in sewage and clinical *E. coli* isolates (Huijbers et al., 2020; Hutinel et al., 2019).

There are two major sampling methods for wastewater surveillance which are time or flow-proportional composite sampling or grab sampling. Composite sampling which involves collecting numerous individual samples in a single sample volume at regular time (or water volume) intervals is considered the most representative method of surveillance in wastewater treatment plants. Automated sampling is a commonly used technology to achieve this, and it involves the use of automated samplers like flow-weighted samplers or continuous composite samplers (Liu et al., 2022). However, autosamplers are expensive, require a constant power supply and require infrastructure modifications before use (Li, Verhagen, et al., 2022; Liu et al., 2022). An alternative cost-effective way could be the use of passive sampling.

Passive sampling involves the spontaneous exchange of a pollutant between the medium being sampled (i.e., wastewater) and a collecting medium (i.e., passive sampler) (Salim & Górecki, 2019). A commonly used passive sampling technique known as the "Moore swab" has been previously used to detect and isolate enteric bacteria in water (Sikorski & Levine, 2020). It has also been used in the collection of viruses such as polio and human norovirus (Matrajt et al., 2018; Tian et al., 2017). The Moore swab involves the use of cotton gauze tied with a string which is suspended in the water and collects micro-organisms over a period of time (Wilson et al., 2022). Apart from gauze, other materials have been used for passive sampling. For instance, Vincent-Hubert *et al*. used various membrane filters such as zetapor, nylon, low-density polyethylene (LDPE) and polyvinylidene difluoride to detect virus (Norovirus, Ostreid herpesvirus type 1) and bacteria (*Vibrio spp*) in seawater (Vincent-Hubert et al., 2017, 2021).

During the SARS-CoV-2 pandemic, passive sampling use in wastewater surveillance of the virus was explored. Several studies assessed the uptake and detection of different sampler materials for the virus. Hayes *et al*. examined the performance of four different sampler materials (cotton gauze, cheesecloth, cellulose sponges, and electronegative membranes) in both bench scale and field experiments. The study reported that cheesecloth and electronegative filter membrane could effectively collect and measure SARS-CoV-2 (Hayes et al., 2021). The use of tampons for passive sampling of the virus in wastewater has also been investigated and demonstrates the ability of the material to retain viral fragments (Bivins et al., 2022; Li, Verhagen, et al., 2022). Various studies have explored the use of gauze, cotton buds, and electronegative membrane placed within a housing for passive sampling of SARS-CoV-2 in wastewater. In these studies, the electronegative membrane had the highest detection capacity followed by gauze and cotton bud (Habtewold et al., 2022; Li, Ahmed, et al., 2022; Schang et al., 2021). In addition, the use of the classic Moore swab deployed in wastewater systems has been reported to be more sensitive than grab sampling in the detection of SARS-CoV-2 at institutional buildings (Corchis-Scott et al., 2021; Liu et al., 2022; Rafiee et al., 2021).

While the passive materials have shown promising capacity in their use for wastewater surveillance, they are prone to disruption due to impaction of solids within wastewater (Schang et al., 2021). A wide range of housing has been used to protect the sampler materials while they are deployed in the wastewater system. Hayes *et al.* designed a hollowed sphere with holes known as the COVID-19 Sewer Cage (COSCa) to house cheesecloth and electronegative membrane. Interestingly, Li *et al.* used hair rollers as a protective casing for tampon material when deployed. Another study by Schang *et al.* also explored different housing casing in various shapes such as colander, matchbox, boat, and torpedo shape. It was reported that the torpedo shaped sampler had lesser ragging rates and clogged openings compared to other shapes of housing (Schang et al., 2021). This 3d printed torpedo shaped sampler has been used extensively in passive wastewater surveillance of SARS-CoV-2 (Li, Verhagen, et al., 2022; Wilson et al., 2022). However, its use in the detection of bacteria is limited.

Although previous studies have reported the use of passive materials within wastewater, most of the recent studies were focused on viruses and the few studies for bacteria used only the gauze material. Therefore, the overall objective of this project was to assess the capacity of different passive materials to detect *E. coli* within wastewater. This would provide insights that could aid optimization of the passive samplers for detection and surveillance of bacteria in wastewater. Wastewater treatment plants are usually assessed for contamination using common indicator bacteria like *Escherichia coli*, enterococci, and coliform bacteria (Da Silva et al., 2006). *E. coli* is also often used as surrogate for Gram negative antibiotic resistant Enterobacterales. Furthermore, *E. coli* survives well in aquatic environment and its detection procedure is well documented and standardized. Therefore, it represents an ideal organism to assesses the effectiveness of using the torpedo shaped passive samplers within wastewater systems to detect bacteria.

The main research questions within this project framework are:

i) Determining the best detachment method of *E. coli* from passive materials.
ii) Comparison of different passive materials.
iii) Correlation between *E. coli* concentration in passive materials and standard autosampler.
iv) Quantification of *E. coli* detected by passive materials at different exposure concentration and time periods.

2.0 MATERIALS AND METHODS

Choice of sampling materials

The torpedo sampler contains gauze, cotton bud and electronegative membranes. However, since the indicator organism (*E. coli*) is negatively charged and more adhesion on positively charged surfaces has been reported (Goulter et al., 2009; Liang et al., 2016; Zheng et al., 2021), this study considered two options to replace the negatively charged membranes. The first option was polyvinylidene fluoride membrane filter (Pvdf). Pvdf has been reported to have a high affinity for *E. coli* (Kumar et al., 2017). The other membrane filter considered is nylon, which has been found to be an efficient membrane in the detection of micro-organisms in seawater (Vincent-Hubert et al., 2021).

2.1 Study design

i) Determining the best detachment method of *E. coli* from the passive materials.

Four independent sampling events were performed for the passive material (gauze, cotton bud, pvdf membrane filter, nylon membrane filter). For each sampling event, four pieces of the passive material of choice (e.g., 4 gauze materials) is placed within the torpedo samplers and deployed in effluent wastewater for 24 hours.

ii) Comparison of different passive materials

2 samplers were deployed in effluent wastewater and retrieved after 24 hours for processing in the laboratory. One sampler contained 1 gauze, 2 nylon membrane filter and 1 bud. The other sampler contained 1 gauze, 2 pvdf membrane filter and 1 bud. The 24-hour sampling was performed once every week for 8 weeks.

In parallel, wastewater samples (24h effluent composite) were collected alongside the passive samplers. During collection, the composite samples were carefully homogenized by manually stirring and a 500ml aliquot was collected and transported at $4^{o}C$ to the laboratory.

iii) Quantification of *E. coli* detected by passive materials at different exposure periods

4 samplers containing gauze, cotton buds, and either a nylon or pvdf filter were deployed in effluent wastewater. The first 2 samplers are retrieved after a 24-hour exposure period. The next 2 samplers are retrieved after a 48-hour exposure period. For each sampling day, a 24-hour effluent wastewater sample was collected. The sampling period lasted for 3 weeks.

iv) Quantification of *E. coli* detected by passive materials at different exposure concentrations

4 samplers containing gauze, cotton bud and either a nylon or pvdf filter were deployed. The first 2 samplers are first placed in effluent (low concentration) for 24 hours and placed in influent (high concentration) for another 24 hours. The 2^{nd} set of samplers are first placed in influent for 24 hours and in effluent for the next 24 hours. In addition, 24-hr composite samples were collected for both influent and effluent on each sampling day. The sampling period lasted for 3 weeks.

2.2 Laboratory analysis

i) Detachment of *E. coli* from the passive materials

After retrieval from the wastewater, the samplers were processed within 24 hours. Each sampler material was placed into a 50ml tube. For each sampling event, the sampler material was processed under 4 conditions to assess the best recovery method. The dilution solution used were Peptone physiological salt (PFZ) and 0.05% Tween. Detachment was achieved either with vortexing for 1 min or with sonication (Branson Sonifier SFX 250, amplitude at 70%) for 1 min 30secs. Therefore, the four conditions were i) PFZ and sonication, ii) 0.05% Tween and sonication, iii) PFZ and vortex, and iv) 0.05% Tween and sonication.

For each condition, samples were filtered through 0.45µm pore size membrane filters. The filtered volumes were 10^{-4}ml, 10^{-3}ml, and10^{-2}ml for gauze, and 0.1ml,1ml, and +/- 8.9 ml (buds and filters). Subsequently, the filters were incubated on Tryptone Bile X-glucuronide agar (TBX) for 4–5 hours at 37 °C, and incubated for 18 hours at 44°C. *E. coli* was quantified by counting the colony forming units on the plate.

ii) Quantification of *E. coli* detected on passive materials

After retrieval from the wastewater, the samplers were dismantled, and each passive material was placed into a 50ml tube. 10ml of PFZ was added to the tube and vortexed for 1 min. For each condition, samples were filtered through 0.45µm pore size membrane filters. The filtered volumes for sampler materials placed in effluent were gauze (10^{-3}ml, 10^{-2}ml, 10^{-1}ml, 1ml and 3ml), bud (10^{-2}ml,10^{-1}ml,1ml and 8.8ml), and filters (0.1ml,1ml and 8.9ml).

The filtered volume for sampler materials placed in influent were equivalent to 10^{-6}ml, 10^{-5}ml, 10^{-4}ml, 10^{-3}ml and 10^{-2}ml for gauze, buds (10^{-4}ml,10^{-3}ml, 10^{-2}ml and 10^{-1}ml), filters (10^{-3}ml, 10^{-2}ml,

10^{-1}ml and 1ml) as achieved through filtration of 1ml of a respective decimal dilution of the original sample in PFZ. Also, effluent filtered volumes were 0.01ml, 0.03ml, 0.1ml, 0.3ml, 1ml and 3ml while influent was 10^{-5}ml,10^{-4}ml,10^{-3}ml,10^{-2}ml and 10^{-1})

Samples were filtered through 0.45μm pore size membrane filters and placed on TBX agar plates. The plates were incubated for 4–5 hours at 37 °C and 18 hours at 44°C

2.3 Data analysis

The data was logged into Microsoft excel and statistical analysis was done in R (version 4.1.2). All *E. coli* counts were log transformed prior to statistical analysis. Descriptive statistics was used to compare *E. coli* counts detected from passive material. A linear model in which the extraction methods and passive materials were considered as fixed effects to assess the quantity of *E. coli* detected was performed. In addition, linear regression analysis was also used to examine the association between *E. coli* detected from passive materials and the standard water samples from autosamplers.

3.0 RESULTS

3.1 Detachment method

E. coli could be quantified in all samples and the type of detachment method affected the *E. coli* counts retrieved from the passive material. The use of peptone physiological salt and vortex was found to be a better recovery method compared to the rest of the detachment methods, according to linear models of the effect of different dilution fluids and detachment methods on *E. coli* detected from passive materials (Table 1).

Table 1: Concentrations of E. coli from passive materials achieved with different detachment methods

Detachment methods	Prediction (log cfu/ passive material)	*P-value*
Peptone physiological salt and Sonication	2.4	
Peptone physiological salt and Vortex	2.9	0.0153*
Tween 0.05% and Sonication	2.5	0.6901
Tween 0.05% and Vortex	2.8	0.0295*

Number of observations: 32

3.2 Quantification of *E. coli* detected by passive materials

The amount of *E. coli* detected from the passive materials was the highest in gauze with a mean concentration of 4.95 log cfu/ passive material while nylon and pvdf had the least *E. coli* detected with a similar mean concentration of 2.3 log cfu / passive material (Table 2). Figure 1 also shows the overall daily average of *E. coli* detected from passive materials in effluent wastewater during the sampling period.

Table 2: Summary statistics of *E. coli* detected from different passive materials

Sampler Material	Arithmetic Mean (log cfu /passive material)	Range (log cfu / passive material)
Gauze	4.95	2.26 – 6.89
Bud	3.68	1.15 - 5.52
Nylon	2.31	0.77- 4.63
Pvdf	2.34	0.58- 4.69

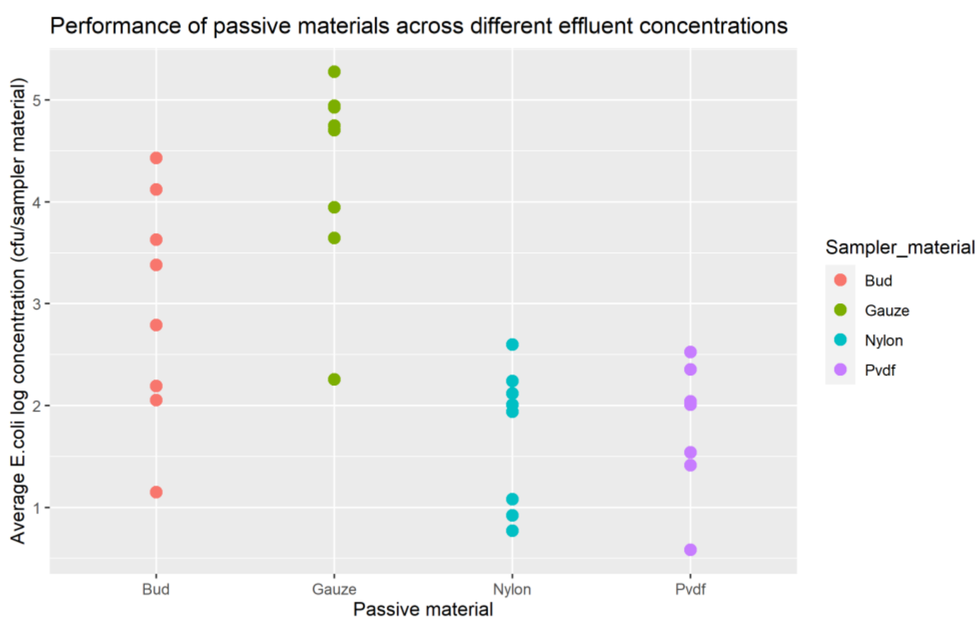

Figure 1: E. coli concentrations detected with different passive materials in 24h effluent samples

3.3 Correlation between *E. coli* detected from passive materials and autosampler

The correlation between the passive materials and the water samples retrieved from 24-hour effluent samples was moderate - good, with the highest correlation found between buds ($R^2 = 0.88$) while the gauze materials were the least correlated ($R^2 = 0.48$), see Figure 2.

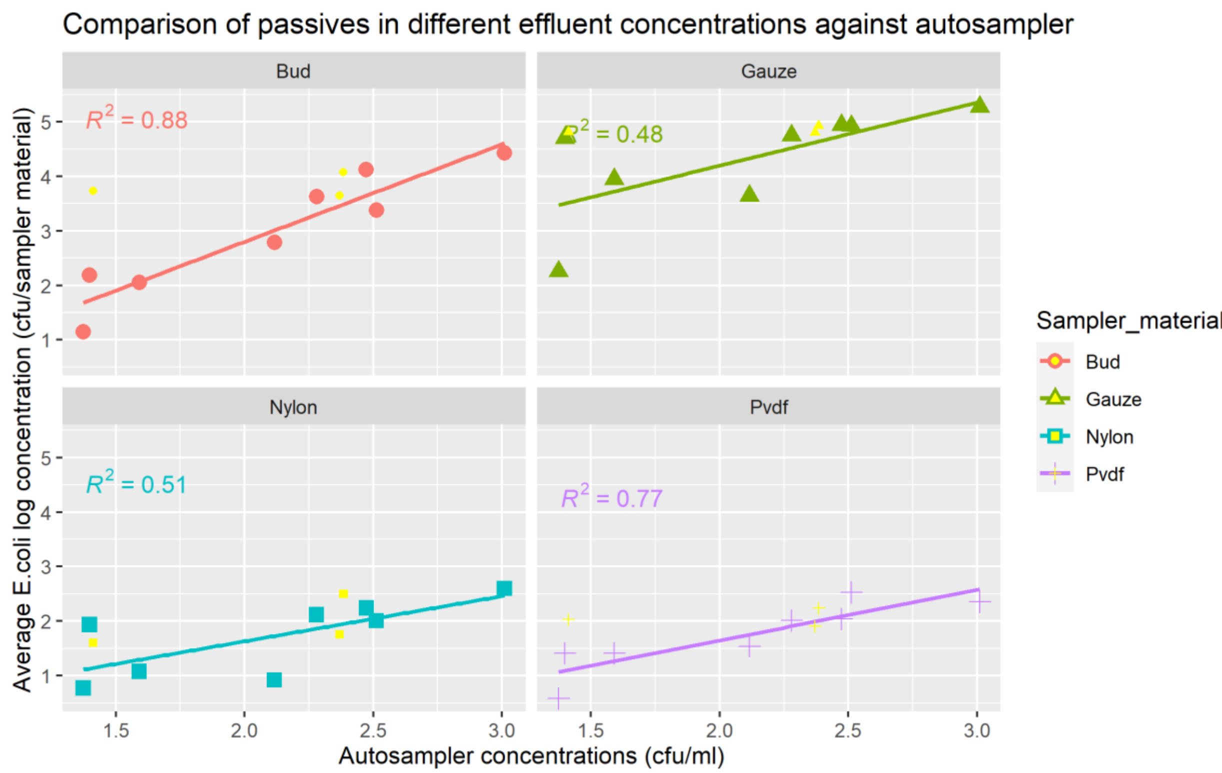

Figure 2: Correlation between *E. coli* detected by use of passive materials and autosamplers
The yellow points show E. coli concentrations for 48-hr exposure periods but were not included in the linear fit.

3.4 Quantification of *E. coli* detected by passive materials at different exposure periods

There were no clear patterns in *E. coli* concentrations detected from passive materials exposed for either a 24-hour exposure period or a 48-hour exposure period. Also, differences in autosampler concentrations between the 2 days were not reflected with the passive materials, see Figure 3. For example, while the *E. coli* effluent concentrations from the autosamplers were similar over 24 and 48 hours for the 3 weeks, buds gave lower counts for the first 24hours in week 2 and 3 but higher counts in week 1. For nylon membrane, *E. coli* counts for the first 24hours were lower in week 2 but higher in week 1 and 3. However, gauze showed relatively similar concentrations over 24 and 48 hours and between sampling events, even if deployed in effluent with varying concentrations.

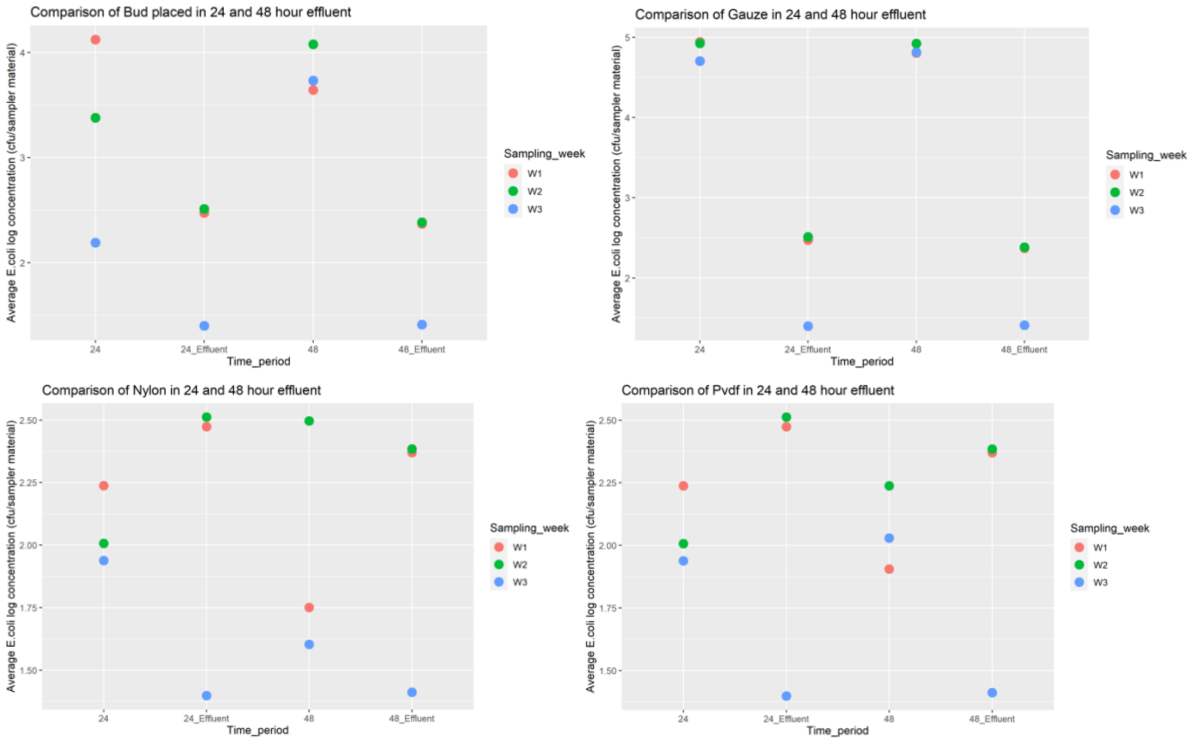

Figure 3: *E. coli* concentrations in 24-hour and 48-hour effluent compared to autosampler.

3.5 Quantification of *E. coli* detected by passive materials at different wastewater concentrations

The amount of *E. coli* detected on passive materials placed in different levels of concentrations over a 48-hour period appears to be relatively similar irrespective of which exposure comes first (i.e., either high or low concentrations) (Figure 4). However, it also appears that the last concentrations of exposure are reflected on the passives. Although when compared to the actual concentration difference between effluent and influents, the difference in the *E. coli* concentrations on the passives is smaller, see Figure 5. To illustrate, the *E. coli* counts of pvdf filters in which the last exposure was influent were higher than the filters placed in effluent last. This pattern can also be seen in the nylon filters and buds, however the difference in gauze materials appear negligible

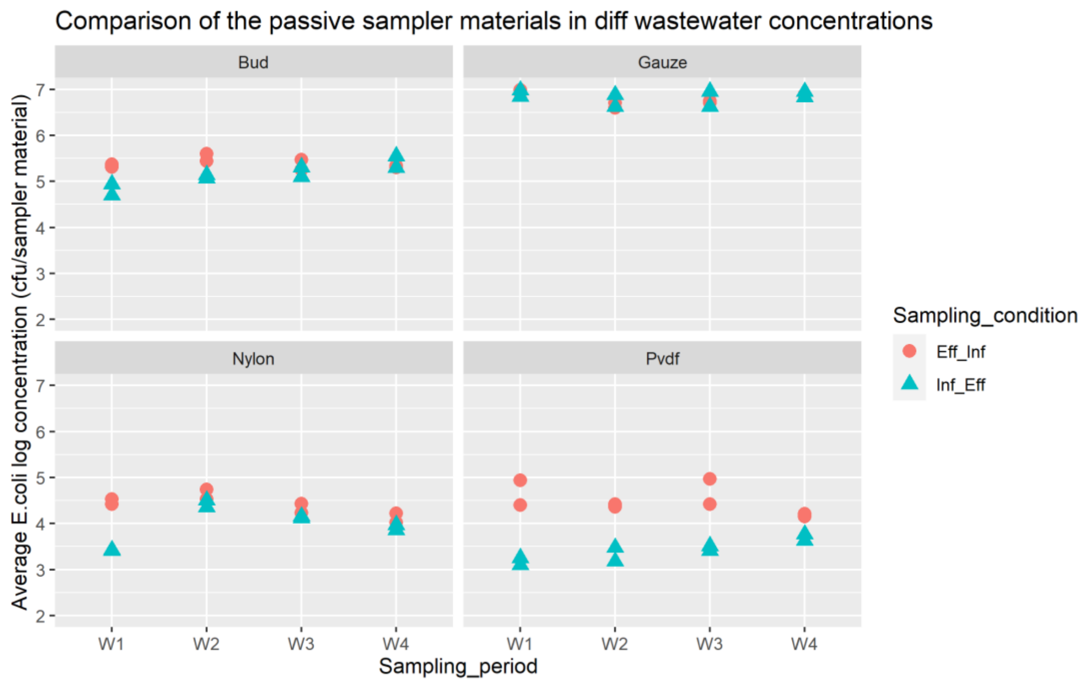

Figure 4: Passive materials exposed to different wastewater concentrations over a 48hour period

Eff-Inf means samplers were first placed in effluent for 24hr and then placed in influent for another 24hr.

Inf-Eff means samplers were first placed in influent for 24hr and then placed in effluent for another 24hr.

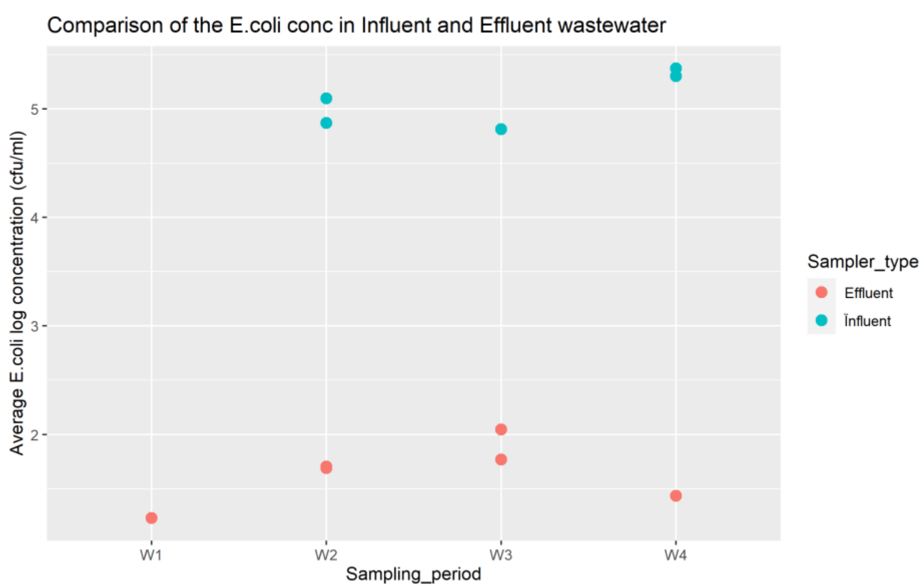

Figure 5: *E. coli* concentration differences between influent autosampler and effluent autosamplers

The results of 48h exposure in switching samples were compared to the results of 24h exposure in effluent. Figure 6 shows the expected relationship between concentrations found in autosamplers and passive samplers when placed in average higher concentrations than effluent wastewater for gauze, bud, nylon and pvdf. The log linear relation between the membrane filters and autosampler concentrations still holds even in higher concentrations, however for buds, lower *E. coli* concentrations are detected compared to estimates based on log linear relations.

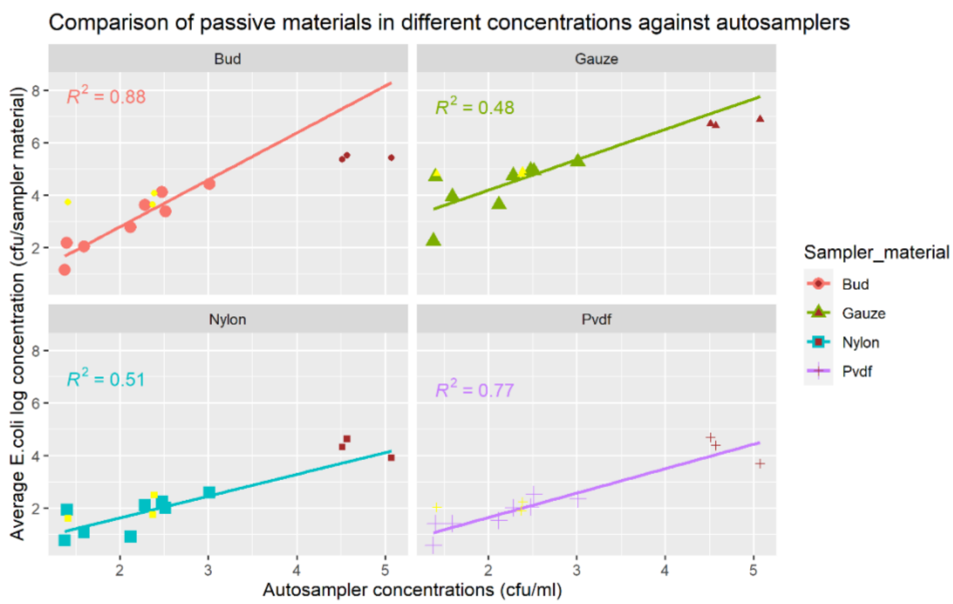

Fig 6: Comparison between E. coli detected on passive materials and autosampler in different concentrations

The yellow points show E. coli concentrations for 48-hr exposure period but were not included in the linear fit.
The brown points show E. coli concentrations for 48-hr exposure period in average high concentrations but were not included in the linear fit.

4.0 DISCUSSION

This study provides insights into the use of different passive materials for the detection of *E. coli* in wastewater. This explorative study is one of the few studies to assess the capacity of different passive materials (gauze, cotton buds, nylon, pvdf) placed within a protective housing to detect *E. coli* in wastewater.

The first aim of this study was to examine the best detachment method of *E. coli* from different passive materials. Peptone physiological salt and vortexing and 0.05% Tween and vortexing were the most promising detachment methods. This highlights that the dilution fluid had a lesser impact than the mechanical detachment methods used. Regarding the mechanical detachment method, vortexing appears to be a better detachment method. It is a convenient inexpensive and widely available technique and has been reported to be effective in dislodging bacteria (Nnadozie et al., 2018; Webber et al., 2015). Therefore, it was chosen as the detachment method for the rest of the study. The use of sonication proved to be less effective by demonstrating a lower yield of *E. coli* from passive materials in this study. Sonication has been used to detach bacteria attached to different surfaces like steel surface, polymers, and wooden surface (Beresford et al., 2001; Bjerkan et al., 2009; Nnadozie et al., 2018). However, the differences in sonication protocols across several studies such as duration of sonication, acoustic frequency and energy makes comparison and standardization difficult.

Furthermore, this study shows that the passive material composition can also have an impact on the quantity of *E. coli* detected on the passive material. Gauze had the highest quantity of *E. coli* detected across different effluent concentrations compared to the buds, nylon or pvdf membrane filters. This may be due to the large surface area compared to other type of passive materials. Also, it is made of interwoven cotton threads which has been reported to have a high absorbency rate (Sikorski & Levine, 2020). In addition, the tendency for retention of solids within the material is high and biosolids in wastewater can act as a vehicular transport for micro-organism which could explain the higher detection rates. However, this tendency for retention may also impact its comparison and correlation with the standard composite concentrations as reflected in the low correlation co-efficient ($R^2 = 0.48$) when gauze is compared with 24h autosampler concentrations.

In addition, buds also appear to perform well in 24h effluent concentrations and had a strong association ($R^2 = 0.88$) with the standard composite samples. However, in comparison to gauze, the quantity of *E. coli* detected was lower in buds. This may be due to the small surface area and the fact that the cotton fibers on the buds are tightly wound around the shaft. This characteristic improves the absorbance capacity of buds but might impede the ability of the buds to release trapped bacteria into the dilution fluid (Moore & Griffith, 2007). However, studies on the use of different types of buds for recovering micro-organisms on different environmental surfaces have reported varied results. For instance, Jansson 2020, reported that recovery of *Listeria* *monocytogenes* and mengovirus from window glass, plastic and wood varied substantially depending on the bud material and surface type (Jansson et al., 2020). In addition, some studies have reported that the swab material, methodology and wetting materials affect bacteria recovery from surfaces (Dolan et al., 2011; Moore & Griffith, 2007). Therefore, within this study it is difficult to determine the exact reason for the reduced detection rate of *E. coli* on buds compared to gauze.

Nylon and pvdf membrane filter had similar detection rates and the least amount of *E. coli* was detected on these membranes. The use of these membranes for passive sampling of microorganisms in water has only occasionally been reported. One study using nylon membrane filter for the detection of *Vibrio spp* in seawater reported that the membrane was effective in detection of the bacteria in seawater compared to LDPE or zetapor filters (Vincent-Hubert et al., 2021). However, it should be noted that the exposure time for the membrane was either 48hours or 15 days which is longer than the duration of exposure in this part of the study. Another interesting finding is that the *E. coli* concentrations on membrane filters maintained their linear relationship between autosampler over a larger range of concentrations compared to other types of passive materials.

Moving on to consider when the passives were exposed to different time durations (i.e., 24 and 48hr period), this study shows that the quantity of *E. coli* detected on the passive materials showed no clear pattern between the two days. Also, the quantity detected on the passive materials was not reflective of the differences found in the composite samples on those two days. However, there were also ample occasions where higher amounts of E. coli were found after 48h as compared to 24h. The results show that the passives concentrations might have more variability in 48-hour exposure period when comparative results to the autosampler is required. Additionally, when the passive materials were exposed to varying levels of concentration within a 48-hour period, there was no strong difference between passives that were first placed in influent for 24hrs then effluent for the next 24hrs and vice versa. This shows that the passive materials can detect *E. coli* even within the variability of high concentrations in wastewater. However, while not showing large differences in E. coli counts depending on the order of placement in wastewater of differing concentrations, buds yielded less E. coli during mixed exposure as compared to exposure in effluent only. This might point to faster saturation levels at high concentrations and might limit bud usage as a passive material for quantitative studies at high concentrations.

In summary, the type of the material to be used for passive sampling in wastewater is highly dependent on the research aims of the study. For example, the high quantity of *E. coli* recovered from gauze material might be sensitive and increase the chances of detecting rare bacteria. Also, the strong linear association of the membrane filters to autosamplers in higher concentrations might improve sampling measurements in high wastewater concentrations.

Strengths, limitations, and future consideration

This study shows that the passive materials can detect *E. coli* in wastewater at different exposure duration and concentration. This can be used for further explorative studies involving *E. coli* within wastewater. In this study, we were able to establish a working protocol for the detachment of *E. coli* from different passive materials.

However, there are some limitations to be considered within the study. In this study, the flow rate of water in which the passives were placed in could not be determined. This might affect the quantitative interpretation of its use within wastewater surveillance. Also, the total number of samples retrieved to assess the capacity of samplers in different durations and concentrations was limited and means interpretation should be approached cautiously.

Future studies that can improve the validation of the passive materials in-situ is recommended. This would further improve the calibration of the passive materials within wastewater. These studies could assess the performance of passive materials to different types of bacteria and different types of wastewaters from various facilities. Also, bench scale experiments that examine how the different sampler materials recover *E. coli* in different water matrices and set durations can help provide more information on the recovery rates, linear uptake, and saturation capacity of the passive materials. This information could be useful for designing sampling approaches and assessing the appropriate exposure duration and ideal deployment times for passive materials to achieve the best results. For instance, if results indicate that certain passives material take certain hours before saturation, then it would be advisable to deploy the samplers within a time duration that one expects peak concentrations of the organism of interest while the absorbent capacity of the passive material is still high.

In conclusion, this study investigated the appropriate method of detachment of *E. coli* from different passive materials placed in wastewater. The use of peptone physiological salt and vortex appears to be a great method for the detachment of *E. coli*. This study also showed that the different passive materials yielded positive detection of *E. coli* within wastewater. The buds showed good linear relationship to the standard autosampler at low concentrations, also the membrane filters had good linear relationship to autosampler at higher concentrations. The *E. coli* concentrations on the passive materials showed more variability over a 48hr period compared to the composite samples. These findings show the potential of passive samplers to be used for wastewater surveillance of anti-resistant bacteria.

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