


RESEARCH ARTICLE

# Unused and expired drug disposal practice and awareness among undergraduate students from pharmacy and other disciplines: Bangladesh perspective

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## Keywords

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## Abstract

**Background:** Most medicine users are not aware of the safe disposal of unused or expired medicines. The aim of this study was to know the current knowledge of the disposal practices of unused and expired medicines among the undergraduate pharmacy and other disciplines studying in universities located in different areas of Dhaka city, Bangladesh. **Methods:** This was a descriptive, cross-sectional survey, conducted through face-to-face interviews using a structured questionnaire. Returned questionnaires were double-checked for accuracy. **Results:** A total of 250 valid questionnaires were returned with a response rate of 100%. Respondents were mainly divided into two categories: pharmacy students (n=150; 60%) and general students (n=100; 40%). Two third of the general students (66%; n=98) and pharmacy students (65.3%; n=66) showed an almost similar response to keeping medicines at home. Overall, the highest common leftover medicines were analgesics (n=181; 27.6%) followed by gastric agents 174 (26.6%). Alarmingly, 85 (34%) respondents usually threw their leftover medicines in the dustbin, however, only very few portions of health science students (8%) have donated their unused medicines to welfare and friends. About 58% of respondents from other disciplines agreed that the necessity of pharmacists counselling people about proper medication disposal is highly required. **Conclusion:** Gaps exist in everyday drug disposal practices, therefore cost-effective pharmaceutical waste management programmes supported by government regulatory authorities and media campaigns are needed. Healthcare practitioners and community pharmacists should offer training to educate patients on standard medicine disposal practices. Findings from this study are hoped to assist in creating awareness about appropriate drug disposal practices in households and trigger interest and attention among policymakers about formulating relevant regulations.

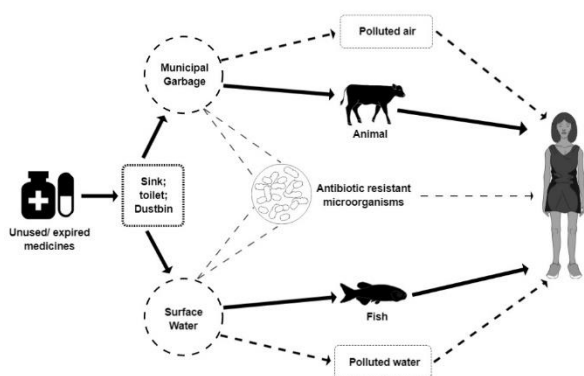
## Introduction

The gross drug consumption in Bangladesh is increasing daily (Nipa *et al.*, 2017). Since Bangladesh has a dense population, a good number of renowned pharmaceuticals and availability of almost all kinds of medicine at a cheap rate, the yearly medicine consumption here must be high compared to any other

country. However, most of the time, these prescribed medicines are left unused due to various reasons like the change of brand names in prescriptions or treatment, side effects of the drugs, and improvement of patient condition resulting in discontinuation of the course of treatment. Reuse of these leftover drugs should be used only under the supervision of medical professionals, especially antibiotics and other

prescription drugs, for safety purposes (Gitawati, 2014). Unused and expired drugs can be detrimental to the environment if they are disposed of in an improper way. Bangladesh, along with many other south and south Asian countries, does not have any official state guidelines or protocols for the disposal of expired and unused drugs (A.Y. Tong, B.M. Peake, & R. Braund, 2011). As a result of this, many people in Bangladesh follow imprudent ways to discard unwanted drugs, such as by throwing those drugs in the dustbin, sink, or toilet, without thinking of the consequences. This method of disposal should be highly discouraged since it directly harms the environmental health and the safety of the people.

When the drugs get disposed of in the dustbin, they later on, deteriorate in the municipal garbage and contaminate the air surrounding it. Animals can get exposed to such medical waste, and when consumed, it not only severely affects the health of those animals but also damages the health of those who consume those animals later on. Thus, improper disposal of drugs can destroy the entire terrestrial food chain in the ecosystem (Figure 1). Non-steroidal anti-inflammatory drug (NSAID) diclofenac has been revealed to induce renal failure in vultures following the ingestion of carrion from cattle treated with this drug (Oaks *et al.*, 2004). Similarly, the unused drugs that are disposed of in the toilet, sink, or dustbin get mixed with the surface water and pollute it. This form of water pollution puts the entire aquatic ecosystem at risk since the ingestion of those drug mixtures is toxic for the water creatures. For example, the trace levels of Ethinyl estradiol, the active component of a common oral contraceptive, interrupt the sexual development and the feminisation of fish (Jobling *et al.*, 2006).



**Figure 1: Consequences of improper disposal of drugs**

Besides, many studies confirm that the presence of antibiotics in water pathways has an impact on the bacteria present in it and may lead to antibiotic

resistance (Costanzo, Murby, & Bates, 2005). This antibiotic resistance in microorganisms may genetically affect humans and marine life in the long term (Wu *et al.*, 2009). Regardless of the disposed of the drug coming in contact with the terrestrial animals, the aquatic creatures, or the microorganisms, it ultimately affects the human as the final step of the food chain. Trace concentrations of pharmaceuticals have previously been spotted in drinking water in the United States and Greece (Heberer, 2002) and cooked seafood (Y. Wang, Yuen, & Ng, 2006). Due to uncontrolled and unsupervised disposal of drugs, a high amount of pharmaceutical wastes flows into the water bodies through sewerage and are also getting absorbed into the land when they are disposed of as solid waste in landfills. This is a great threat to the safety of the environment and food sources (Daughton, 2007).

Necessary steps such as determining the gross amount of unused drugs that are disposed of via the water systems or the landfill, specifying guidelines for the proper disposal of the drugs by the policymakers, and implementing them strictly all over the country can be taken to prevent further damage to the environment caused by improper disposal of drugs. Moreover, mass awareness toward the safe disposal of unused and expired drugs must be created to save the ecosystem. In Bangladesh, no significant and comprehensive research work has been done to investigate the drug disposal practice among undergraduate students from pharmaceutical sciences and other disciplines.

Currently, medical waste management and disposal is a less discussed topic in Bangladesh because of the lack of awareness about the accidental poisoning and environmental damage that can be caused by the improper disposal of household drugs. Improperly disposed of drugs can contaminate the environment and pose a risk to water, air, agricultural products, and the food chain and even harm animals/ livestock. Therefore, studies have been conducted throughout the world about this issue to find policy solutions. However, this study so far is the first in-depth, comprehensive study which thoroughly explores the drug disposal practice and awareness among undergraduate students from the pharmacy and other disciplines in Bangladesh.

## Methods

### Study design

It was a descriptive, cross-sectional study, and a pre-validated structured questionnaire consisting of 16 questions was used to conduct the survey. This study was conducted in the top 10 private universities in

Bangladesh and was conducted according to the guidelines of the Declaration of Helsinki. The study population was chosen, irrespective of gender, between the ages of 20 and 26 years, which included students from private universities who were studying Bachelor in Pharmacy, Engineering and Business Administration.

### Questionnaire design

The questionnaire was structured after reviewing a number of literature and divided into two sections. The first section of the questionnaire was comprised of personal information, including age, sex, subject of study, a common class of leftover drugs, and reasons to keep medicines at home. The questions in the second were designed to assess the practices, knowledge and attitudes of the participants concerning unused and expired medication disposal, including what they did with leftover solid and liquid medicines, did they know the standard drug disposal method, whether they were aware of the environmental hazard of improper drug disposal, what according to them can be the best way to create awareness, and importance of pharmacists to create awareness regarding proper disposal practices. Finally, the questionnaire was reviewed by an expert for content validity.

### Data collection and analysis

Participants only who were willing to participate in the survey were provided with a questionnaire (in English) to fill up. The individuals assigned to collect the response, at first, explained the purpose and importance of the study to the potential respondents. After completion, questionnaires with responses were collected for statistical analysis.

At first, the collected data were fed into the Microsoft Excel spreadsheet dataset and then transferred to Statistical Package for Social Science (SPSS) version 23.0 for analysis using descriptive statistics (descriptive, crosstab and chi-square). Responses with multiple answers were defined in multiple response sets before descriptive statistical analysis.

### Results

A total of 250 respondents participated in this study. All the approached 250 individuals responded, and none declined. Hence respondent's response rate was 100%. Of the 250 respondents, 147 (58.8%) were men, and 103 (41.2%) were women. Respondents were between 20-26 years of age. Around three-quarters of the population (74%) were in the age group 20–23 years, 14.4% were below 20 years, and 10.4% were 24–25

years, 1.3% were exactly 26 years old. Respondents were mainly divided into two categories: pharmacy students (n=150; 60%) and general students (n=100; 40%).

**Table I: Demography of study population**

Variable and categories	Number of responses (%)	
<b>Classification</b>		
Pharmacy students	150	(60%)
General students	100	(40%)
<b>Group</b>		
Pharmacy	150	(60%)
Engineering	53	(21.2%)
Business Administration	47	(18.8%)
<b>Age</b>		
20 years	36	(14.4%)
21 to 23 years	185	(74%)
24 to 25 years	26	(10.4%)
26 years	03	(1.2%)
<b>Gender</b>		
Male	147	(58.8%)
Female	103	(41.2%)

### Practice toward medicaments storage and disposal

Data in Figure 2 showed that unused medicines were stored by more than half of the respondents, 164 (65.6%). Moreover, two-thirds of the general students (66%; n=98) and pharmacy students (65.3%; n=66) showed an almost similar response to keeping medicines at home. Amidst both groups, the highest reason for storing medicines was in case they are needed later (74.7%). Common reasons for stocking medicines are illustrated in Figure 3. One in four (25%) general students confirmed that they did not know how to dispose of drugs. On the other hand, only a few (5%) future pharmacy students were also not aware of it.

Overall, the highest common leftover medicines were analgesics (n=181; 27.6%), followed by gastric agents 174 (26.6%) Figure 4. A significant difference was noticed in the frequency of unused antibiotics by 44 (44%) general students and 30 (20%) future health care providers. In both categories, nearly half of the respondents (n=124; 49.6%) stored their unused medicines until they expired (Figure 5). Out of 250 respondents, 85 (34%) respondents usually threw their leftover medicines in the dustbin; however, only very few portions of health science students (8%) and other students (7%) have donated their unused medicines to welfare and friends. There was no significant difference observed within the response of both categories.

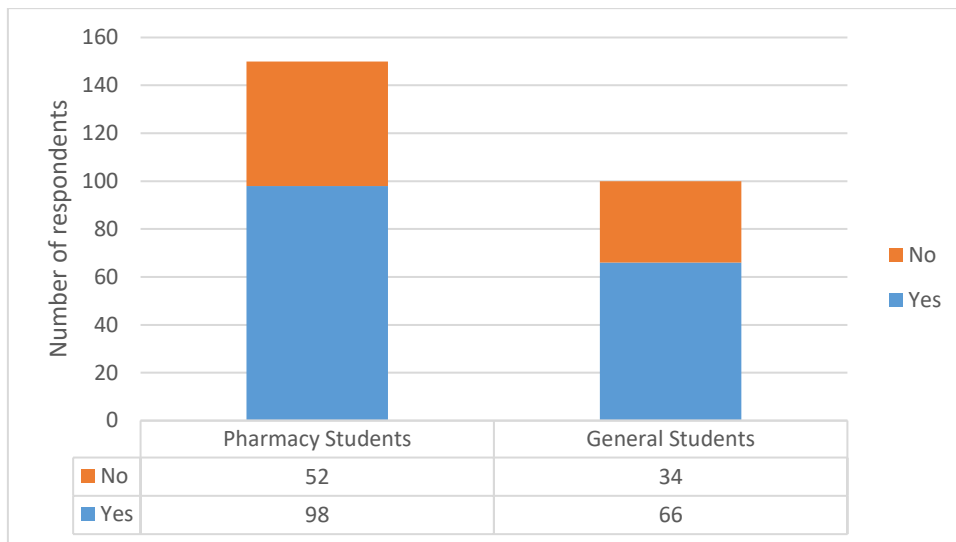


Figure 2: Tendency of storing leftover medications

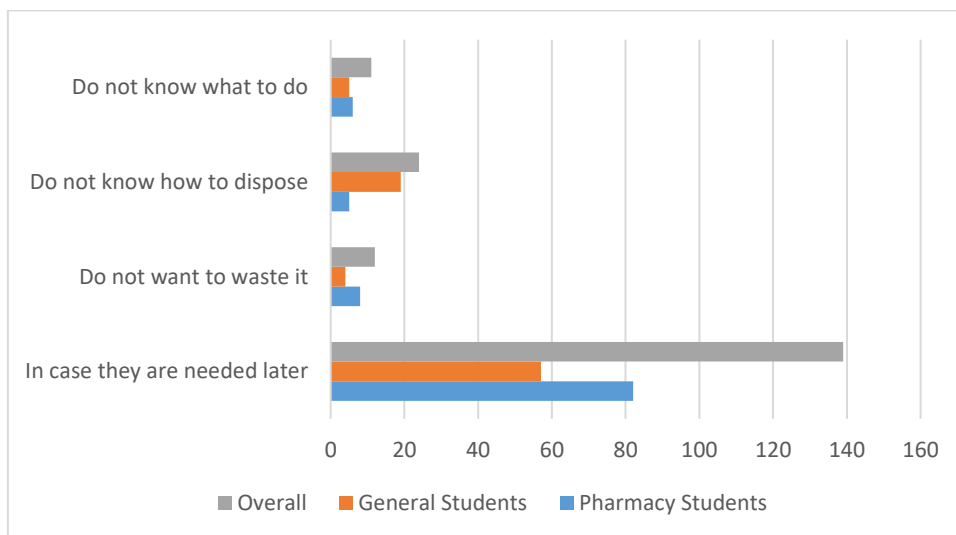


Figure 3: Common reasons for storing medicines

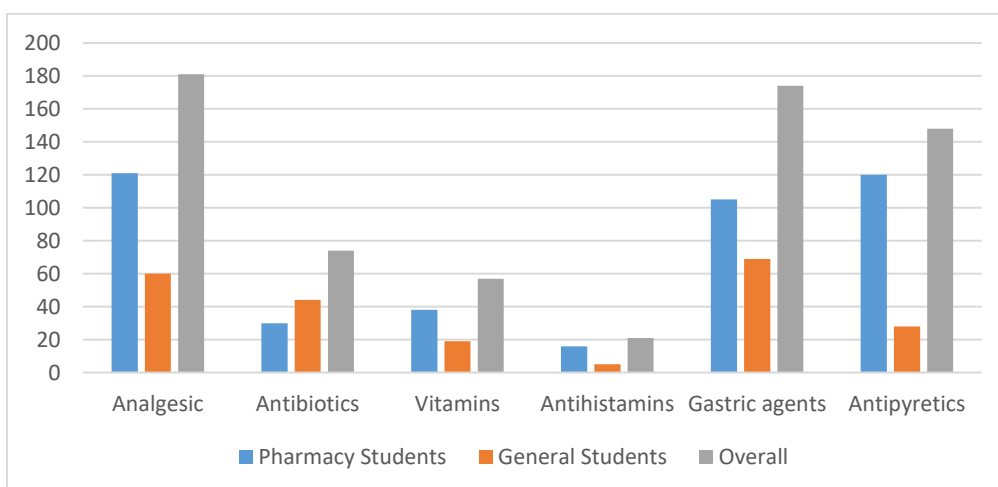
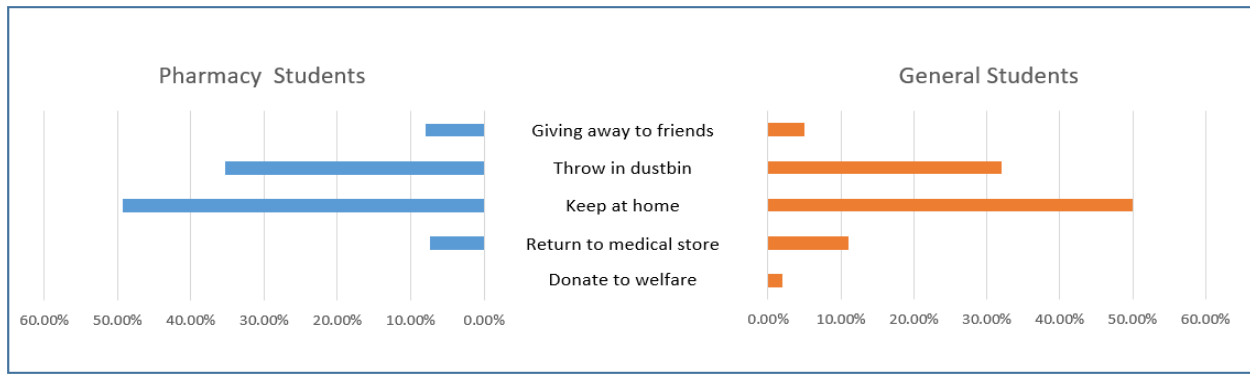


Figure 4: Frequency of different common classes of leftover medicines at home

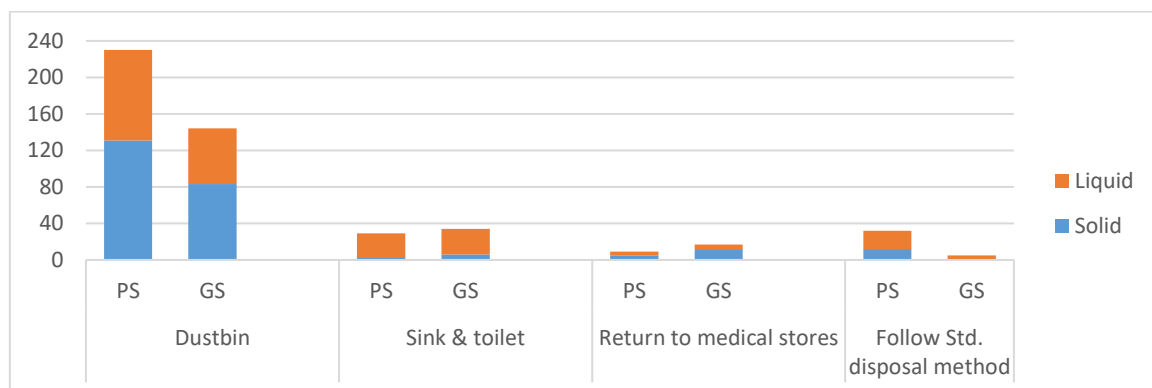
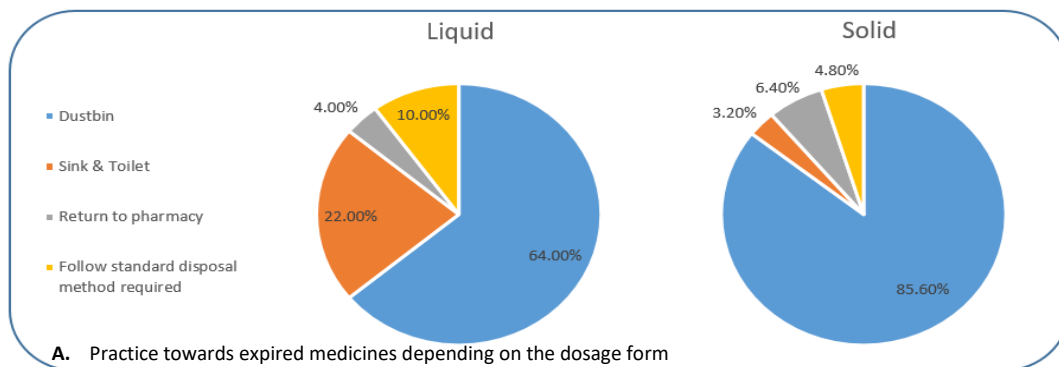


**Figure 5: Fate of leftover medicines by the respondents**

Findings of this study also revealed that 86.5% (n=214) and 64% (n=160) of the total respondents frequently disposed of expired solid and liquid medications, respectively, via dustbin. In the case of expired liquid medications, a significant number of 55 (22%) people have chosen flushing medicines in the sink and toilet as the second common method. Compared to general students (n=0, 0%; n=5; 5%), upcoming health professionals (n=12, 8%; n=20, 13.3%) have shown sufficient knowledge to dispose solid and liquid medicines respectively following required standard disposal method. The influence of different dosage forms (solid/liquid) does not produce a significant

difference in the disposal practice of both pharmacy and general students (Figures 6a & 6b).

This study portrayed (Figure 7) that general students (40%, n=40) were less concerned about the true practice of medicine disposal at home compared to pharmacy students (32%; n=49). However, approximately one in two respondents of both general students (48%; n=72) as well as pharmacy students (46%; n=46), dumped their medicines as they are. Even though compared to other students (10%, n=10), students of pharmacy discipline (16.7%, n=25) were more likely to crush medicines before discarding them.



**Figure 6: Prevalence of expired solid and liquid medicines disposition via different methods**

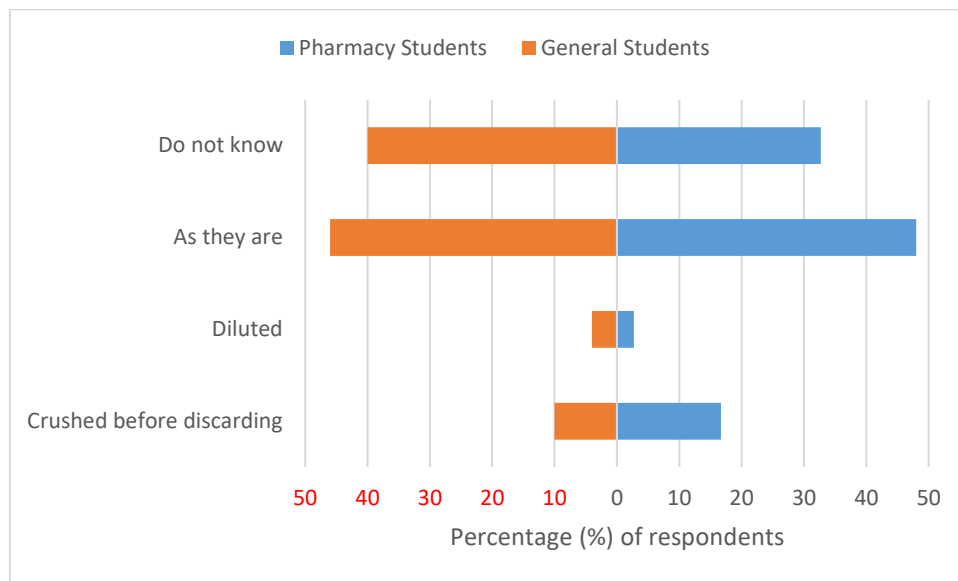


Figure 7: Practice toward discarding medicines at home by both Pharmacy students and general students

**Knowledge about medicine disposition**

A noteworthy amount of students (87.2%; n=218) confirmed that they do not have any drug take-back programme in their society. There was no notable difference observed between the responses of general students and medicine-related subjects students (Table II). In response to a question regarding whether they had heard of the standard disposal method, 46% of

pharmacy students and 21% of general students answered positively. Similarly, three-quarters of the pharmacy students (72.7%; n=109) and half of the other students' respondents (48%; n=48) were aware of the consequences of improper medicament disposition. Using the Pearson Chi-square test, a statistically significant amount of differences in knowledge ( $p < 0.01$ ) and awareness ( $p < 0.01$ ) were noted among the two categories.

Table II: Respondents' knowledge and attitudes concerning unused/expired medication disposal

Questions	Options	Number of responses (%)
Do you have any drug take-back system in your society?	Yes	32 (12.8%)
	No	218 (87.2%)
Have you ever heard about the standard drug disposal method of medicines?	Yes	91 (36.4%)
	No	159 (63.6%)
Do you know about the environmental hazards because of the improper disposal of medicines?	Yes	157 (62.8%)
	No	93 (37.2%)
How could be the hazardous effect of unused and expired medicines minimised or controlled?	Lowering the number of prescribed medicine by the doctor	47 (12.6%)
	Donating the unused medicines	49 (13.2%)
	Ensuring patient complete their courses through proper counselling by pharmacists	124 (33.3%)
	Establishing a drug take-back system to dispose of drugs safely	139 (37.4%)
	Making mass awareness about the hazardous effect of unused and expired drug	13 (3.5%)
What do you think would be the best source of awareness about proper drug disposal for our society?	Newspaper	20 (7.0%)
	TV & internet	157 (55.3%)
	Seminar & workshop	17 (6.0%)
	Pharmacist	55 (19.4%)
	Doctor	35 (12.3%)

### Attitudes towards the awareness of medicine disposition

Table II depicts different methods that could be used to reduce the prevalence of expired medicines. As a solution to this grave issue, 37.4% of the population have selected establishing a drug take-back programme, 33.3% answered improving patient compliance through proper counselling by pharmacists, 13.2% suggested donating the unused medicines for welfare, 12% said reducing the number of medicine prescribed by the doctor and only 3.5% proposed enhancing awareness about the impact of improper drug disposal.

To create awareness about proper drug disposal practices, more than half of the students (55.3%)

suggested the internet and TV as the most popular method, and nearly 20% of people recommended assigning pharmacists as the second most demanding means. Besides that, fewer amounts of people have chosen doctors (12%), newspapers (7%) and seminars (6%). The necessity of pharmacists for counselling people about proper medication disposal is measured on a scale of one to four, which is portrayed in Figure 8. Scale four denotes that pharmacists are extremely required, and scale one represents that pharmacists are not required at all. It is obvious from the data where it reflects that almost 80% of the people encouraged recruiting pharmacists, and 20% said maybe pharmacists could resolve this; on the contrary, around 10% answered pharmacists are not required in this matter.



Figure 8: Responses regarding the requirement of counselling by pharmacists for proper medicament disposal

### Discussion

This study has proved that the majority of people aged between 20-26 years old (Table I) (40%) are unaware of the safe disposal methods of drugs (Figure 3). This study's findings demonstrated that knowledge and awareness about leftover household drug disposal among undergraduate students in Bangladesh are alarmingly low. It has been seen that respondents keep analgesics, antibiotics, vitamins, antihistamines, gastric agents, and antipyretic drugs, which may lead to a public health hazard (Van Der Geest, 1982). This survey has shown that the highest common leftover medicines in Dhaka city were analgesics (n=181; 27.6%), followed by gastric agents 174 (26.6%) (Figure 4). Whereas another study in Kabul, the capital of Afghanistan, showed that the majority of leftover drugs were antibiotics (46.5%) (Bashaar *et al.*, 2017).

A very recent survey was conducted throughout the households of Dhaka metropolis in Bangladesh, which shows that only 94% of participants claimed to be taking medicine after being prescribed it by the doctor, and 32% of respondents bought antibiotics, sedatives, and sex stimulants without prescription (Begum *et al.*, 2021). The findings of that study regarding drug disposal practice generate distressing similarities with this study. In Dhaka Metropolitan, almost 67% of respondents have no idea about drug disposal (Begum

*et al.*, 2021). Similarly, 63.6% of the respondents, which comprises only undergraduate students in pharmacy and other disciplines, have never heard about the standard drug disposal method (Table II).

This scenario was also similar in some developed countries years ago. A study conducted in the United States found that 98% of the respondents kept unused drugs in the household. However, following the Drug Enforcement Administration (DEA) establishment, a National Prescription Take-Back day is being celebrated to collect unused medications (Glassmeyer *et al.*, 2009). The systematic medicine take-back programme is also satisfactory in Australia (Guirguis, 2010). In this survey, 49.6% of students in both categories, from the pharmacy and other departments, have stored their unused or expired drugs in their homes (Figure 2). In New Zealand, a third party is appointed by pharmacists to collect and destroy unused and expired drugs (A. Tong, B. Peake, & R. Braund, 2011). Only 8% of the respondents donate their unused medicines to welfare and friends (Figure 5). Students from the pharmacy department donate their medicines to welfare more often because they know the usage indications of those drugs.

However, in developing and underdeveloped countries, the situation is even worse. In a study in Ethiopia, even though most participants (58%) are aware of the

hazards resulting from improper drug disposal and prefer FDA (Food and Drug Administration) and WHO (World Health Organization) disposal methods (75%), this does not comply with existing Ethiopian practice. About 21% were used to keep unused medicine at home (Atinafu *et al.*, 2014). Another study in India revealed that 96% of consumers threw away expired medication, and 73% were discarded in household trash with other garbage (Manocha *et al.*, 2020).

Another study also confirmed that medication waste management had become a major challenge to the healthcare sector in Bangladesh (Hassan *et al.*, 2008). The study in Ethiopia showed that throwing into the trash (16.4%), flushing into the toilet (13.3%), and throwing into the environment (10.4%) were the common practices of drug disposal (Atinafu *et al.*, 2014), whereas, in this study, it has been seen that 22% of the participants' flush down liquid drugs and 3.20% flush down-solid drugs into the sink and toilet. Besides this, 64% of liquid drugs and 85.6% of solid drugs have been thrown into the dustbin by undergraduate students in Bangladesh (Figure 6). The study in Kabul, Afghanistan showed that even though 98% of the respondents are aware of improper drug disposal practices' negative environmental consequences, around 77.7% of them discarded unused medicines directly in household garbage (Bashaar *et al.*, 2017).

A study among 143 families from urban and rural settings together in Serbia showed that the most common drug disposal method was throwing into the garbage (80.3%), even though half of them were aware of the harmful consequences of their exposure to the environment (Kusturica *et al.*, 2015). Another study in Saudi Arabia found that 28% of participants were used to keeping unwanted and unused drugs in the home, and only one-third of them returned unused and unwanted drugs to the pharmacy (Akici, Aydin, & Kiroglu, 2018).

It is high time strict legislation to stop such malpractices are enforced. Mailing or returning leftover medicines to the pharmacy, using a disposal kiosk, carefully trashing them, and flushing out liquid medicines can be some disposal methods (L.S. Wang, Aziz, & Chik, 2021). The manufacturers should use tamper-resistant boxes to allow the return of unused medicines in a pharmacy. Not only medicines but also medical apparatuses, like syringes, saline bottles, ampules, vials, and tubes, must be disposed of safely and immediately after use (Marwa *et al.*, 2021). It is not practical for a developing country like Bangladesh to dispose of pharmaceuticals at high temperatures (i.e. above 1200°C) for incineration along with adequate emanation control (Jha, Shankar, & Palaian, 2021).

The majority of the participants in this study (37.4%) agree that establishing a drug take-back system for safe disposal would be the most suitable way to prevent the hazardous effects of improper drug disposal methods. Since there is no medicine take-back programme available in Bangladesh, these simple steps to dispose of most medicines in the household trash can be followed: 1). Mix the medicines (do not crush tablets or capsules) with an unpalatable substance such as kitty litter or used coffee grounds (Al Rawwad *et al.*, 2021); 2). Place the mixture in a container such as a zip-top or sealable plastic bag and throw the container in your household trash; 3). Before throwing out a medicine container, such as a pill bottle, remember to scratch out all the personal information on the prescription label to make it unreadable (Iabu *et al.*, 2013).

Before implementing any intervention, people should first be educated on the necessity of the disposal of pharmaceuticals. The pharmacies already manage drugs and can recommend what type of disposal method is the most appropriate. They can stockpile drugs and dispose of them as a community service. Some regions may have special government services that offer to take back and dispose of drugs. Sometimes, either a pharmacy or a government service will provide a way to dispatch drugs to a special drug disposal facility (Kral *et al.*, 2021). Flushing pills into the toilet can trigger drug pollution, but this might be a justified way to rapidly dispose of hazardous drugs. We should flush strong pain medicines such as OxyContin down the drain as soon as they are no longer needed to avoid accidental poisoning (Iabu *et al.*, 2013).

Following prescriptions properly is a prerequisite to following a dosage regimen and ensuring the rational use of drugs. Ensuring this will minimise the piling up of unused medications to a greater extent. Several practical approaches could improve the malpractices of improper drug disposal. These are: creating awareness among mass people by involving all kinds of media (print, electric, and social), policy-making by the appropriate body for legislation and regulation, involving climate activist groups or institutions enabling safe leftover medicine disposal campaigning, the inclusion of specific information and warnings regarding safe disposal of medicine in the label and secondary packages, encouraging of returning medicine to the pharmacies, and leftover medicine collection by crowdfunding, etc. A total of 58% of the respondents think recruiting pharmacists for the safe disposal of drugs is extremely important (Figure 8).

This is because pharmacists study the safe usage and disposal methods of drugs extensively throughout their education. Pharmacists raising consciousness on the detrimental effects improperly disposed of drugs have



on the environment, the ecosystem, and the health of everyone can be saved in an exponential way (Lv, Liu, & Lay, 2021). This investigational study was done to explore the current disposal patterns and awareness of household medicines among these students in Bangladesh. The study tried to generate preliminary evidence on leftover and household medicine disposal patterns and knowledge of the consequences of it. Findings from this study are hoped to assist in creating awareness about appropriate drug disposal practices in households and trigger interest and attention among policymakers about formulating relevant regulations.

A checklist of consulting requirements for future researchers; Nowadays, drug disposal practices have become very crucial in terms of many aspects. The good thing is research regarding expired and unused drug disposal practices are happening, though scarcely. So, there are some areas to develop the ideas from a researcher's point of view concerning proper drug disposal practices.

### **Limitations**

In this study, the authors only included the thoughts of the pharmacy students and some general students. But, could not conduct a survey on the mass people's thoughts, that is, from which perspective they actually see this issue of drug disposal practices. Also, this study didn't discuss the government initiatives, for instance: social campaigns and public health campaigns that can be taken to reduce the inappropriate drug disposal practices. The government can make a monitoring body to practice their role regarding this drug disposal issue. Disposal of Unwanted Medication Properly (DUMP) campaign was launched in New Zealand, and in Canada, a drug disposal programme was initiated. These countries can be role models for Bangladesh. The DGDA- 'Directorate General of Drug Administration,' can take place to reduce the improper practices of drug disposal. DGDA have to control and proctor the power of a drug in the country. They can set a regulatory body regarding disposal practices of unused and expired medicines as well as they can limit some rules and laws. There can be a bidirectional combination between DGDA and drug consumers. Moreover, the above-mentioned campaigns can also alter the disposal practices in a moderate way. So, it can be another aspect for future researchers on how government can take proper action and initiatives to decrease the improper practices of drug disposing of.

Improper drug disposal practices can be very much hazardous to the environment as there are so many routes of drug administration. By default, the environment, plants, and animals can be harmed, and

the ecosystem can be altered. The European Environment Agency (EEA) identified concerns over the environmental impact of pharmaceutically active substances as a significant emerging issue. But, the authors didn't discuss how a healthy environment can be altered, and the behaviour of the environment can differ because of wrong disposal practices of unused and expired medicines. So, this could be a vital point from a future researcher's perspective concerning environmental issues related to drug disposing of practices. In the 21st century, recycling is very famous and has become a trend as well. Recycling (reclamation)-"Drug mining," such as hospital reclamation of highly toxic drugs from excreta and other wastes, could be pursued and expanded.

The drug-producing company and pharmaceutical industry's role in the disposing practices haven't been written in this research paper. Companies can play a significant role, including awareness of the packaging materials concerning proper drug disposal practices. So, the public can learn more ideas from the medicine's packaging. This factor can carry out further research as well. If further research happens in terms of unused and expired drug disposal practices from Bangladesh's perspective, the above-mentioned facts can be useful and can be taken under consideration by future researchers.

### **Conclusion**

In developing countries like Bangladesh, the scenario is very distressing. The findings of this study reflected the present scenario regarding the methods of medicament waste disposal practised by the undergraduate students of pharmacy and other disciplines from different universities located in Dhaka city, Bangladesh. This study observation concluded that organising seminars by government-regulated drug administration, environmental scientists, physicians and pharmacists might play a pivotal role in raising awareness in the community based on the slogan "*give your unused/expired medicines a proper home*".

Moreover, doctors, nurses and hospital and community pharmacists can also be enlightened through campaigns and workshops. Along with these, awareness through electronic media and newspapers appeared to be preferable. At university undergraduate levels, some general education courses on safe drug disposal practices can be a good option to educate the nation's future leaders. Last but not least, immediate extensive researches on environmental pharmacy are mandatory to conduct because the most practised disposal method has the ability to cause disastrous

environmental effects in Bangladesh like in other densely populated countries.

### Conflict of interest

The authors have not declared any conflict of interests.

### Acknowledgement

The self-funding of the research team has carried out this research.

### Informed consent statement

Informed consent was obtained from all subjects involved in the study.

### Authors' contribution

All authors have equal contributions to this manuscript.

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