Supplementary data

Questionnaire S1: Questionnaire used in the study.

Exploring the Challenges to Antimicrobial Stewardship: Leftover Antibiotics, Self-Reuse, and Disposal Practices among Residents of Karachi, Pakistan.

Thank you for considering participation in this survey!

We aim to gather insights into the leftover, self-reuse, and disposal practices of antibiotics among residents of Karachi. The purpose of this survey is to understand common practices related to antibiotic use, which will contribute to a broader effort to promote health awareness and encourage responsible medication use.

Participation in this study is entirely voluntary. You are free to choose whether to participate, and you may withdraw at any point without any consequences. Your responses will remain confidential and will only be used for research purposes.

The survey will take approximately 5-10 minutes to complete. If you have any questions or concerns about this survey, please feel free to contact us.

By proceeding, you indicate that you have been informed about the purpose of this study and consent to participate.

Section 1: Demographic Information

1) Name: _____

2) Age
18-27
28-37
38-47
48-57
58-67
>67

3) Gender
Male
Female
Other/Prefer not to say

4) Residence
Urban
Rural

5) Education
No formal education
Primary education (1-5 class) Secondary education (6-10 class)
Higher Secondary Education (11^{th} and 12^{th}) Undergraduate
Bachelor's degree
Master's degree Doctorate

6) Marital status
Single
Divorced
Widow
Married (No kids)
Married (with kids)
Extended family

7) Employment
Student
Employed (medical-related)
Employed (Non-medical related)
Unemployed
Retired

Section 2: Practices Related to Antibiotic Use and Leftovers

8) When was the last time you took prescribed antibiotics?
Last month Last 3 Months
Last 6 Months Last year
More than a year

Don’t remember
Never

9)Did you complete the full course of antibiotics as prescribed?
Yes
No

10)If no, why did you not complete the full course?
Felt better/symptoms resolved
Experienced side effects
I forgot to take doses
Medication was too expensive

11)Do you typically have leftover/unused antibiotics prescribed by your doctor?
Yes
No

12)What are the reasons for having leftover/unused antibiotics?
Symptoms improved before completing the course
Experienced side effects
I forgot to take doses
The doctor switches on another antibiotic
The doctor advised me to stop taking them Purchased more quantity than prescribed

13)Have you ever used leftover/unused antibiotics without consulting a doctor?
Yes
No

14)If yes, why did you use leftover antibiotics without consulting a doctor?
Wanted to save time
Save money
The same medicine successfully resolved symptoms previously
The same medicine worked for my friend/family/relatives with a similar complaint
Did not think it was necessary to consult a doctor

15)Is it okay to buy the same antibiotics without a Dr consultation if you’re sick and they helped you get better when you had the same symptoms before?
True
False
Don’t know

16)Have you ever given leftover antibiotics to family/friends/relatives because you were used to treat the same illness
Yes
No

17)What do you think about the reuse of these leftover antibiotics
Save time
Save money
Treat symptoms early
Complicate the disease
Don’t know

Section 3: Disposal Practices
18)Are you aware of proper disposal methods for leftover and expired antibiotics?
Yes
No

19)What do you usually do with leftover antibiotics?
Keep them for future use
Dispose of them in the trash (dustbin)
Flush them down the toilet
Return them to a pharmacy or take-back program
I don’t dispose of medications
Donate to others

20)What do you usually do with expired antibiotics?
Keep them for future use
Dispose of them in the trash (dustbin) Flush them down the toilet
Return them to a pharmacy or take-back program I don't dispose of medications
Donate to others

21)When you receive antibiotics from the pharmacy, are you properly counseled or educated on how to dispose of these?
Yes
No
Don't know

22)Are you aware of state or federal guidelines for safely disposing of prescribed antibiotics?
Yes
No
Don't remember

23)Do you believe improper antibiotic disposal negatively impacts public health and the environment?
Yes
No
Don't know

24)Do you know the Pharmacy's take-back programs for leftover/expired medications?
Yes
No
Don't Know

25)Would you use a safe place to dispose of medications if one was available?
Yes
No
Don't know

26)Would you give this information to others to use a safe disposal place if available?
Yes
No
Don't know

27)Effective methods to raise community awareness regarding medication disposal
Deliver clear instructions while patients receive medications
Patient education by healthcare experts Awareness through Social media
Other techniques

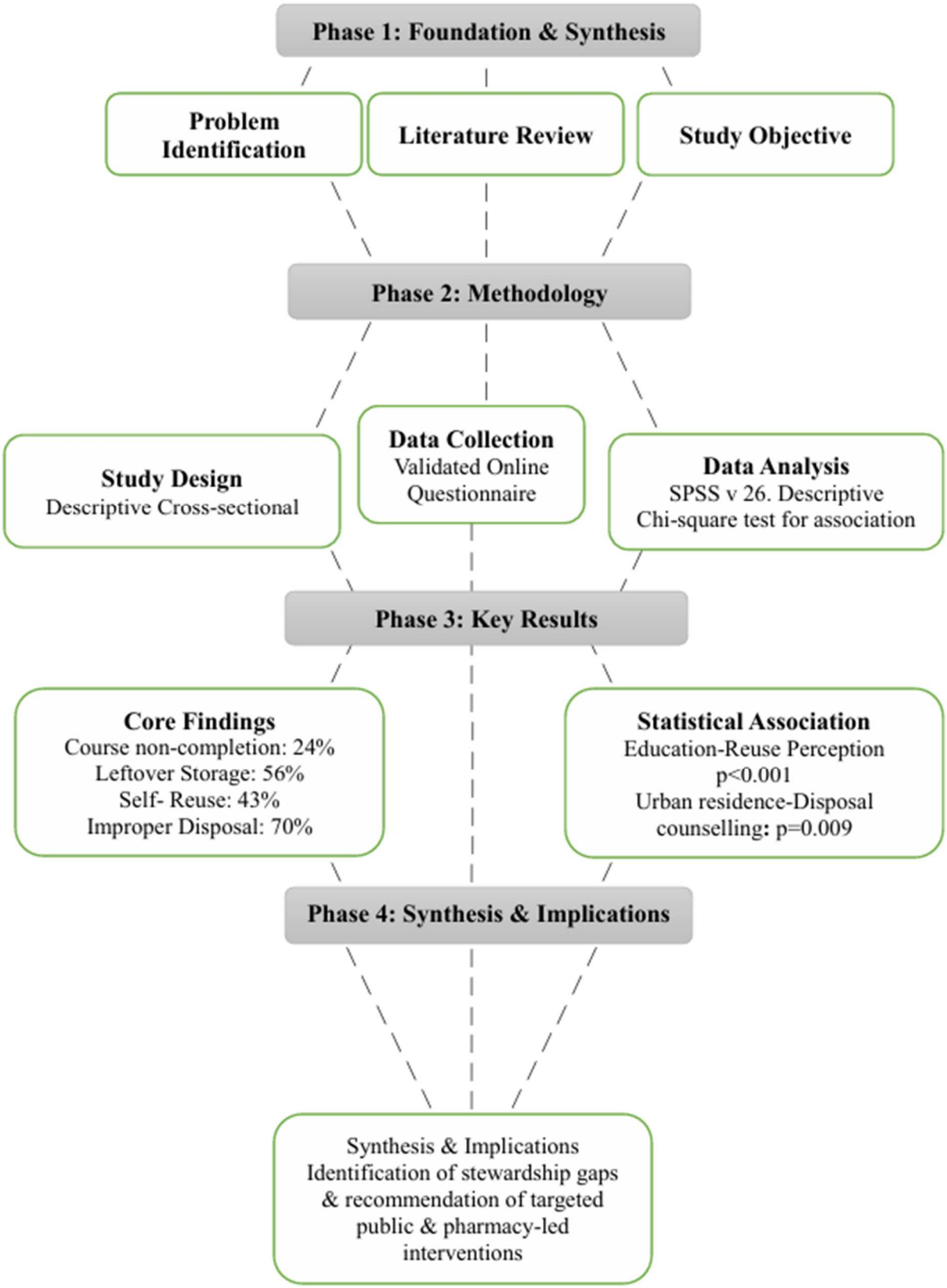

Fig. S1: Research flow chart

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	(Challenges to antibiotic stewardship: A cross-sectional study on self-reuse and disposal practices of leftover antibiotics)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1	(A descriptive, cross-sectional study was conducted using a validated online questionnaire targeting adults (≥18 years) residing in Karachi. A total of 385 responses were collected via non-probability sampling. The findings highlight concerning patterns in antibiotics storage, self-use and disposal practices in Karachi. These patterns suggest a need for public educational, pharmacist-led interventions and improved access to medication take-back programs to promote safer practices and reduce AMR risk)
		Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	1	(Antimicrobial resistance (AMR) is a growing global health threat, primarily driven by the inappropriate use of antibiotics and unsafe disposal practices. In developing countries like Pakistan, the misuse of leftover antibiotics through self-medication and improper disposal remains a poorly addressed public health concern).
Objectives	3	State specific objectives, including any prespecified hypotheses	1 and 2	(This study aimed to describe the prevalence, patterns and associated factors of self-reuse and disposal practices of leftover antibiotics among residents of Karachi, Pakistan and to identify gaps affecting antibiotic stewardship)
		Methods		
Study design	4	Present key elements of study design early in the paper	2	(Cross-sectional online survey-based study).
Setting	5	Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up,	2 and 3	(Following approval from the Institutional Review Committee (IRC), the data collection was conducted from 10/02/2025 to 03/06/2025, exploring the

		and data collection		challenges to antibiotic stewardship, leftover antibiotics, self-reuse and disposal practices among residents of Karachi, Pakistan).
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls N.A *Cross-sectional study*—Give the eligibility criteria and the sources and methods of selection of participants	3	Adults (≥18 years) residing in Karachi, who reported the use of antibiotics in the past and were willing to complete an online questionnaire regarding their practices related to leftover antibiotics, self-reuse and disposal, were included. Both males and females were eligible to participate).
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed: N.A *Case-control study*—For matched studies, give matching criteria and the number of controls per case: N.A		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders and effect modifiers. Give diagnostic criteria, if applicable	3	(Descriptive statistics were used to summarize sociodemographic and behavioural variables. All completed questionnaires were reviewed for completeness; responses with more than 20% missing data in primary outcome variables were excluded from the analysis (n=5). For variables with minimal missing responses (<5%), missing values were handled using pairwise deletion in chi-square analysis. Associations between categorical variables were examined using Pearson's chi-square test, with Fisher's exact test applied where expected cell counts were below 5. The tool/questionnaire used in the study was developed and validated through expert focus groups, face and content validation and a Cronbach's alpha test. The Sample size was calculated using the WHO sample size calculator. Random sampling technique was employed).

Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*3*	(The participants were recruited through non-probability convenience sampling using online platforms, including social media (Facebook, WhatsApp, Instagram, Viber), email distribution list and professional networks of the research team. The survey was hosted on Google Forms and settings were configured to allow only one response per email address to prevent duplicate submissions).
Bias	9	Describe any efforts to address potential sources of bias	3	(In order to minimize bias, the study employed a validated questionnaire and rigorous methodology. The tool/questionnaire used in the study was developed and validated through expert focus groups, face and content validation and a Cronbach's alpha test. The Sample size was calculated using the WHO sample size calculator. Random sampling technique was employed. Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS), with $p<0.05$ considered significant. Data collection and analysis were blinded to minimize observer bias. There was no selection bias, measurement bias, or confirmation bias. All these measures ensured the study's robustness and minimized bias).
Study size	10	Explain how the study size was arrived at	3	(A non-probability convenience sampling method was employed due to feasibility and access constraints. The sample size of 385 was estimated using the WHO sample size calculator for population surveys, assuming a 50% prevalence of leftover antibiotics practices (to maximize sample size), a 95% confidence interval and a 5% margin of error).

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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why N.A		
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	3	(Descriptive statistics, Pearson's chi-square with Fisher's exact test)

		(*b*) Describe any methods used to examine subgroups and interactions N.A		
		(*c*) Explain how missing data were addressed No missing data were found		No missing data were found
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed: N.A *Case-control study*—If applicable, explain how matching of cases and controls was addressed: N.A *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy: N.A		
		(*e*) Describe any sensitivity analyses: N.A		
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up and analysed	4 and 5	(385 individuals were eligible, included, completed follow-up and analysed).
		(b) Give reasons for non-participation at each stage: N.A		
		(c) Consider use of a flow diagram	13	Supplementary figure S2: Research flow chart.

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4	The characteristics of the study participants have been described, including demographic variables (age, gender, marital status), educational level, employment status and socioeconomic status (SES). These variables also represent potential confounders and relevant exposure-related factors for the study outcomes, as detailed in Table 1 of the paper).

		(b) Indicate number of participants with missing data for each variable of interest: N.A		
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount): N.A		
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time: N.A		
		Case-control study—Report numbers in each exposure category, or summary measures of exposure: N.A		
		Cross-sectional study—Report numbers of outcome events or summary measures	5 and 6	The outcome events and summary measures have been reported in terms of the prevalence of key behaviors, including leftover antibiotic availability, self-reuse without medical consultation and unsafe disposal practices. Additionally, statistically significant associations were identified between educational attainment and antibiotic-related beliefs and behaviors. Socioeconomic differences in receiving counselling on antibiotic disposal were also observed. These findings are presented as proportions and percentages, with corresponding statistical significance reported where applicable.
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included: N.A		
		(*b*) Report category boundaries when continuous variables were categorized: N.A		
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period: N.A		

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions and sensitivity analyses: N.A		
Discussion				
Key results	18	Summarise key results with reference to study objectives	9,10 and 11	Approximately 24% of participants did not complete their prescribed antibiotic course, predominantly because of symptom resolution. Over half (56%) reported retaining leftover antibiotics and 43% admitted to reusing them without professional consultation, largely due to previous successful outcomes. A significant association was observed between educational level and perceptions of the safety of antibiotic reuse ($p < 0.001$). Disposal practices were suboptimal, with 70% discarding expired antibiotics in household trash and only 8% utilizing pharmacy take-back services. Counselling on proper disposal varied significantly by socioeconomic status (SES) ($p = 0.009$), with the highest rate reported among low SES participants (37.5%), but overall awareness of disposal programs remained low.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12	Despite the study having an adequate sample size, participants being predominantly young, highly educated females, recruited via non-probability sampling, might have limited the generalization of the results to the broader, more diverse population of Karachi and wide confidence intervals for smaller subgroups (e.g., lower educational levels) reflect limited precision in those estimates. As this study used self-reported data, which is susceptible to social desirability bias, it may lead to underreporting of inappropriate practices, such as self-medication or antibiotic sharing. Despite these limitations, this study highlights key knowledge gaps and behavioral patterns in antibiotic stewardship, offering preliminary insights that could inform future public health interventions.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of	12	The results are consistent with evidence from similar low- and middle income settings. The observed associations, particularly with education and
		analyses, results from similar studies and other relevant evidence		socioeconomic status, are indicative but not conclusive, highlighting the need for further studies to confirm these findings and guide targeted interventions.
Generalisability	21	Discuss the generalisability (external validity) of the study results: N.A		
Other information				

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based:	12	There was no funding.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies: N.A

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/ and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.