RESEARCH ARTICLE

Burden of drug-resistant pulmonary tuberculosis in Pakistani children: A cross-sectional study [version 1; peer review: 1 approved with reservations]

Ghulam Shabbir Laghari¹, Zahid Hussain², Lavina Khemani³,
Syed Zohaib Maroof Hussain⁴, Uzair Yaqoob ⁵

¹Department of Pediatrics, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan

²Department of Pediatrics, National Institute of Child Health, Pakistan, Karachi, Pakistan

³Dow University of Health Sciences, Karachi, Pakistan

⁴Aga Khan University Hospital, Karachi, Pakistan

⁵Jinnah Postgraduate Medical Centre, Karachi, Pakistan

V1 First published: 27 Mar 2019, 8:344
<https://doi.org/10.12688/f1000research.18507.1>
 Latest published: 26 Apr 2019, 8:344
<https://doi.org/10.12688/f1000research.18507.2>

Abstract

Introduction: The incidence of drug-resistant tuberculosis (TB) is rapidly increasing worldwide. Children in high TB burden countries are rapidly being reported to be affected by multidrug-resistant TB (resistant to isoniazid and rifampicin). The aim of this study is to evaluate the pattern of drug sensitivity among children suffering with TB.

Methods: Known cases of pulmonary TB, with sputum smear positive even after two months of compliance to 1st line anti-tuberculous therapy were included after gaining informed consent. Specimens used for drug sensitivity analysis were either sputum or bronchoalveolar lavage. Patient age, gender, history of TB contact, and duration of treatment were also recorded. Data was entered and analyzed using SPSS v.22.

Results: There were 32 male (64%) and 18 female (36%) children in the study. Their mean age was 12.84 ± 2.54 years. History of household TB contact was positive in 29 (58%) children. Among 1st line anti-tuberculous therapy, ethambutol and streptomycin were most sensitive ($n=44$; 88%). Rifampin was least sensitive ($n=17$; 34%). There were 32 (64%) children with multidrug-resistant tuberculosis (MDR-TB). A positive history of household TB contact (either resistant or non-resistant) was seen to have a statistically significant impact on incidence of MDR-TB (p value=0.03)

Conclusion: Pediatric drug-resistant TB is a rising concern. Awareness programs on national and international levels are needed to educate the general population regarding the importance of preventing TB household contact, especially amongst children.

Open Peer Review

Approval Status

	1	2
version 2
(revision)	view	view
26 Apr 2019

version 1
27 Mar 2019	view	

1. **H. Simon Schaaf** , Stellenbosch University, Cape Town, South Africa

2. **Nazia Jamil** , University of the Punjab, Lahore, Pakistan

Any reports and responses or comments on the article can be found at the end of the article.

Keywords

pediatric tuberculosis, multi-drug resistant tuberculosis, isoniazid, rifampicin, household T contact, Pakistan

This article is included in the **World TB Day** collection.

Corresponding author: Uzair Yaqoob (ozair_91393@hotmail.com)

Author roles: **Laghari GS:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Hussain Z:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Khemani L:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Hussain SZM:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Yaqoob U:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

Copyright: © 2019 Laghari GS *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Laghari GS, Hussain Z, Khemani L *et al.* **Burden of drug-resistant pulmonary tuberculosis in Pakistani children: A cross-sectional study [version 1; peer review: 1 approved with reservations]** F1000Research 2019, 8:344 <https://doi.org/10.12688/f1000research.18507.1>

First published: 27 Mar 2019, 8:344 <https://doi.org/10.12688/f1000research.18507.1>

Introduction

Globally, there are approximately 67 million children suffering from *Mycobacterium tuberculosis* (MTB) infection. The incidence of isoniazid (INH) mono-resistance has been estimated to be 5 million, and 2 million for combined isoniazid and rifampicin (RIF) resistance. In 2014 alone, an estimated 850,000 children developed pulmonary tuberculosis with 25,000 multidrug-resistant cases¹. The statistics surged drastically, and in 2017 1 million new cases of paediatric TB were reported². Adding to the current poor trajectory there have also been reports of extensive drug resistance in paediatric pulmonary tuberculosis, with almost 100,000 such cases reported¹.

In TB patients, drug resistance results from spontaneous genetic mutations in the MTB genome. The risk of genetic mutation increases with increasing bacterial load, explaining why resistance is more commonly seen in adult cavitary TB, which has large bacilli load. In children the more common reasons of drug resistance are transmission of a resistant bacillus and previous treatment with anti-tuberculous therapy (ATT). Other factors that predispose to drug-resistant TB include inappropriate drug regimens, monotherapy, and drug non-adherence³.

Pakistan is among the top 20 TB-endemic countries, which share 84% of global TB burden and 87% of MDR-TB burden, according to the World Health Organization (WHO)². Though, there have been various studies highlighting the incidence of MDR-TB in Pakistani adults⁴, and some studies also included children; we couldn't find any study from Pakistan that discussed the incidence of paediatric MDR-TB in particular. The aim of this study is to assess the pattern of sensitivity to 1st line and 2nd line ATT among Pakistani children (≤ 18 years).

Methods

A prospective, cross-sectional study was conducted from 1st July 2018 to 31st Dec 2018 in the Department of Paediatrics, Civil Hospital, Jamshoro. Known cases of pulmonary tuberculosis being followed up at the outpatient TB clinic were recruited. The inclusion criteria included children <18 years with a working diagnosis of pulmonary TB who had been taking 1st line ATT for two months but still had sputum smears (or sputum culture) positive for MTB. For children less than five years old, informed consent was taken from their parents/guardians. For children of age five years or above, informed consent from the parents/guardians and assent from the children was taken. Children who had become negative for MTB on sputum smear or culture with 1st line ATT, indicating response to these drugs, were not included. Children who were sputum positive but also non-compliant to their medications (compliance checked from their TB dosage card) were also excluded. Follow up patients in the TB clinic whose parents/guardians did not consent or children older than 5 who did not assent to participate were also excluded.

For culture and sensitivity, either sputum sample was utilized or bronchoalveolar lavage specimen (in cases of no sputum production). The samples were not specifically taken for this research, but were a part of their standard management, hence, no

additional burden was placed on the participants of the study. *Mycobacterium* was isolated from the specimens by using Lowenstein-Jensen medium and Mycobacterium Growth Indicator Tube (MGIT) medium (Becton Dickinson, Franklin Lakes, NJ, USA). BACTEC NAP test (Becton Dickinson) was then performed on the isolated mycobacterium to differentiate MTB from other mycobacteria. Drug susceptibility testing was then done using an agar proportion method on enriched Middlebrook 7H10 medium (BBL Microbiology Systems, Cockeysville, MD, USA) following the standard laboratory protocols of the Civil Hospital, Jamshoro. Concentrations used for every drug was: isoniazid (INH) 0.2 μ g/ml, rifampicin (RIF) 1 μ g/ml, ethambutol (EMB) 5 μ g/ml, and streptomycin (SM) 2 μ g/ml and 10 μ g/ml. For pyrazinamide (PZA) sensitivity, BACTEC 7H12 medium was used with pH 6.0, at 100 μ g/ml (BACTEC PZA test medium, Becton Dickinson). Strains which were not susceptible to INH and RIF were termed as MDR strains. MDR-TB strains were then tested for susceptibility to 2nd anti-tuberculosis agents: capreomycin 10 μ g/ml, ofloxacin 2 μ g/ml, ethionamide 5 μ g/ml, and kanamycin 6 μ g/ml.

A brief questionnaire (See Extended data⁵) was generated which included patient demographics such as age, gender, history of TB contact, and duration of treatment. Data was entered and analyzed using SPSS Version 22.0. Armonk, NY: IBM Corp. Mean $\pm$ standard deviation (SD) was calculated for continuous variables such as age and duration of treatment. Frequency and percentages were calculated for all other variables including drug sensitivity.

Results

The study was completed by 50 children. There were 32 male (64%) and 18 female (36%) children in the study. Their mean age was 12.84 ± 2.54 years with the youngest child being 7 and the oldest 18. The demographic profile of these patients is shown in Table 1 (data at patient level is available as Underlying data⁵).

The sensitivity pattern of 1st line ATT is shown in Table 2. Ethambutol and streptomycin were most sensitive ($n=44$; 88%). RIF was least sensitive ($n=17$; 34%). There were 32 (64%) children with combined sensitivity to INH and RIF and 18 (36%) children were multidrug-resistant i.e., combined resistance to

Table 1. Demographics profile of the patients.

Patient characteristics	Frequency n (%)
Age in years (mean $\pm$ SD)	12.84 $\pm$ 2.54
Duration of treatment in months (mean $\pm$ SD)	3.34 $\pm$ 2.27
Gender	
Male	32 (64%)
Female	18 (36%)
History of TB contact	
Yes	29 (58%)
No	21 (42%)

TB – Tuberculosis

Table 2. Susceptibility pattern to first line anti-tuberculosis agent on sputum samples.

DRUG	SENSITIVITY n (%)	RESISTANCE n (%)
STREPTOMYCIN	44 (88%)	6 (12%)
ISONIAZID	20 (40%)	30 (60%)
RIFAMPICIN	17 (34%)	33 (66%)
ISONIAZID + RIFAMPICIN (MDR)	32 (64%)	18 (36%)
PYRAZINAMIDE	25 (50%)	25 (50%)
ETHAMBUTOL	44 (88%)	6 (12%)

MDR- multidrug-resistant

INH and RIF. Other than MDR cases, and among the first line drugs used alone, RIF showed the highest isolated resistance (n=33; 66%).

Of the 18 MDR cases, 10 (55.6%) were boys and 8 (44.4%) were girls. Their mean age was 14.01 ± 1.50 years with the youngest of aged 12 and oldest aged 15.

The sensitivity pattern of second-line line ATT is shown in Table 3. Kanamycin and capreomycin showed 100% sensitivity. Ethionamide was sensitive in 47 (94%) children and ofloxacin was sensitive in 38 (76%) children.

A positive history of household TB contact (either resistant or non-resistant) was seen to have a statistically significant impact on incidence of MDR-TB as seen in Table 4.

Discussion

The incidence of drug-resistant TB among children is a global health concern. Public health specialists must pay keen attention to this issue in order to prevent unnecessary mortalities. Pakistan is already a high TB burden country. Poor detection, diagnosis, and management of TB, along with unchecked household contact of children with tuberculosis patients, has markedly contributed to the rising incidence of MDR-TB among both adults and children in Pakistan⁶. This study reported 66% of children to be mono-resistant to RIF, 36% to be MDR, and although no case of extended drug resistance was seen, 24% of children tested positive for fluoroquinolone resistance.

Comparatively, in a Pakistani study conducted in 2010–14, of all the MDR-TB cases in the study, only 1.6% were aged 0–14⁴. In another survey from 2013–14, household contacts of 209 diagnosed cases of MDR-TB were screened. It was seen that 378 of 1463 contacts (26%) were children aged 0–15. Of these, 11 children were symptomatic for TB, were tested, and 4 cases of TB were diagnosed from these children, all of which were MDR⁷. This study highly reinforces the impact of household TB contact on the development of MDR-TB in children, which has also been highlighted in our study. In another study, with 62% individuals resistant to all first-line agents, ofloxacin resistance was among 52.7%; which is relatively low in the current study (24%)⁸.

Table 3. Susceptibility pattern to second line anti-tuberculosis agent on sputum samples.

DRUG	SENSITIVITY n (%)	RESISTANCE n (%)
KANAMYCIN	50 (100%)	0 (0%)
CAPREOMYCIN	50 (100%)	0 (0%)
ETHIONAMIDE	47 (94%)	3 (6%)
OFLOXACIN	38 (76%)	12 (24%)

Table 4. Impact of household tuberculosis (TB) contact history on incidence of multidrug-resistant (MDR)-TB.

Household TB contact history	Incidence of MDR-TB n (%)	No incidence of MDR-TBn (%)	P value
Positive (n=29)	14 (48.3%)	15 (51.7%)	0.03
Negative (n=21)	4 (19%)	17 (81%)	

This study highlights the prevailing situation of anti-tuberculosis resistance in Pakistani children and their predisposing factors. It emphasises the need to protect the children from TB infected persons. This study has its limitations too. It was based in one institute only which is in the rural part of Pakistan. The study cannot be generalised to the national status of Pakistan. Multi-center studies all across Pakistan must be conducted to completely understand the current status of anti-tuberculosis drugs resistance in Pakistan among both children as well as adults. Studies should also be conducted to evaluate disease outcome in these patients.

Conclusion

Drug-resistant TB, especially in the pediatric population, is a public health concern. Awareness programs on national and international levels are needed to educate the masses regarding importance of preventing TB household contact especially among children. Long term studies should be conducted to study the prognosis of children with MDR-TB and deduce strategies to prevent drug resistance.

Ethical approval and consent to participate

The study was assessed and approved by the Institutional Review Board of Civil Hospital, Jamshoro (IERB: 18-679) with informed consent taken from all participants.

Data availability

Underlying data

Figshare: Burden of drug-resistant pulmonary tuberculosis in Pakistani children. <https://doi.org/10.6084/m9.figshare.7823741.v4>⁵

This project contains the following underlying data:

- PTB.sav (Antibiotic sensitivity analysis data)
- Data Dictionary.spv (Data dictionary for underlying data)

Extended data

Figshare: Burden of drug-resistant pulmonary tuberculosis in Pakistani children. <https://doi.org/10.6084/m9.figshare.7823741.v4>⁵

This project contains the following extended data:

- Questionnaire.docx (Study questionnaire)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Grant information

The author(s) declared that no grants were involved in supporting this work.

References

1. Dodd PJ, Sismanidis C, Seddon JA: **Global burden of drug-resistant tuberculosis in children: a mathematical modelling study.** *Lancet Infect Dis.* 2016; **16**(10): 1193–201.
[PubMed Abstract](#) | [Publisher Full Text](#)
2. **Global Tuberculosis Control.** [Internet]. World Health Organization; 2018 [cited 2 March 2019].
[Reference Source](#)
3. Shah I: **Multidrug-resistant tuberculosis in children.** *Pediatr Infect Dis J.* 2012; **31**(9): 970–2.
[PubMed Abstract](#) | [Publisher Full Text](#)
4. Akhtar AM, Arif MA, Kanwal S, *et al.*: **Prevalence and drug resistance pattern of MDR TB in retreatment cases of Punjab, Pakistan.** *J Pak Med Assoc.* 2016; **66**(8): 989–3.
[PubMed Abstract](#)
5. Yaqoob U: **Burden of drug-resistant pulmonary tuberculosis in Pakistani children.** *figshare.* Fileset. 2019.
<http://www.doi.org/10.6084/m9.figshare.7823741.v4>
6. Ahmad AM, Akhtar S, Hasan R, *et al.*: **Risk factors for multidrug-resistant tuberculosis in urban Pakistan: a multicenter case-control study.** *Int J Mycobacteriol.* 2012; **1**(3): 137–42.
[PubMed Abstract](#) | [Publisher Full Text](#)
7. Qadeer E, Fatima R, Haq MU, *et al.*: **Yield of facility-based verbal screening amongst household contacts of patients with multi-drug resistant tuberculosis in Pakistan.** *J Clin Tuberculosis Mycobacterial Dis.* 2017; **7**: 22–7.
[Publisher Full Text](#)
8. Ahmad N, Javaid A, Sulaiman SA, *et al.*: **Resistance patterns, prevalence, and predictors of fluoroquinolones resistance in multidrug resistant tuberculosis patients.** *Braz J Infect Dis.* 2016; **20**(1): 41–7.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Peer Review Status: ?

Version 1

Reviewer Report 11 April 2019

<https://doi.org/10.5256/f1000research.20251.r46354>

© 2019 Schaaf H. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

H. Simon Schaaf

Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

Data on the burden or incidence of drug-resistant tuberculosis (DR-TB) in children is sparse, as bacteriological confirmation of tuberculosis in children is challenging. This study did not set out to determine the incidence of DR-TB in Pakistani children in their setting, but rather to evaluate DR-TB in children (0-<18 years) who still had a positive smear microscopy for acid-fast bacilli or cultures for *Mycobacterium tuberculosis* positive after 2 months of adherent first-line antituberculosis treatment. The authors found a very high rate of drug resistance in this highly selected patient group (previously treated, mainly adolescents), it is difficult to extrapolate this data to other settings even in the same country. The reviewer has the following comments:

Major comments:

1. Abstract: The way in which the drug susceptibility test (DST) results are presented is confusing and the abstract data regarding MDR-TB is different from the manuscript (32 cases in abstract vs 18 in manuscript text/table). I suggest change results to: rifampicin resistance was highest at 33/50 (66%), and resistance to streptomycin and ethambutol were the lowest (6/50; 12%). Please provide the correct data for MDR-TB.
2. Abstract conclusion: These conclusions cannot be made from this study. This is a biased study of highly selected, mainly older children for DST. What could be said is that with the selected method used to identify mainly older children with drug resistance, the yield for drug-resistant TB was high.
3. Introduction 1st paragraph: There is a clear difference between TB infection and TB disease. In this paragraph, these two entities are confused. It should clearly state that the numbers presented refer to infection and not disease, e.g. it is estimated that 5 million children are infected with INH mono-resistant *M. tuberculosis* strains, 2 million with MDR strains and (in the last line) 100,000 are infected with XDR strains. These are not TB cases! Also, there was not 1 million child TB cases "reported" – this was only estimated – only about a third of this number were reported cases.
4. Introduction 2nd paragraph: "Children" in this study covers a wide age range from 0-<18 years. Especially in young children <5-10 years of age (before they develop cavitory adult

type disease) transmission of drug-resistant strains is the main reason for DR-TB. Once they become adolescents, with adult-type disease, incorrect prescribing and/or poor adherence to medication become much more common reasons for developing DR-TB. Previous treatment in children returning with DR-TB is often not because of developing resistance, but because DR-TB was not diagnosed at initial presentation. In young children, because of the low bacillary load, a two-month negative culture does not always mean a good response – they may fail later during treatment or relapse after completion of first-line treatment. This is likely one of the reasons why so few young children were identified in this study.

5. Methods: In resource limited settings, plans sometimes need to be made to identify the highest risk group(s) for DR-TB, which the authors likely did in this study. Unfortunately the highly selected group of children studied means that nothing can be said about the incidence of DR-TB in this community – other than maybe that DR-TB is unlikely to be higher than this study's findings in this setting. It is unfortunate that children with poor compliance to treatment who were still positive (AFB smear or culture) after two months were excluded, as this group has a high risk of developing resistance in the first two months of treatment. The authors should define “non-compliant” to medication.
6. It would also be helpful for the readers to place the studied cases and DR-TB cases identified during this study into context if they knew how many children in total were on TB treatment at the time of the study in this setting, how many parents/children did not consent/assent to the study and how many were excluded due to non-compliance. Are these numbers available?
7. Results: Presenting the results in the text as “most/least sensitive” and “combined sensitivity” is confusing – it even confused the authors themselves (see abstract vs text). It is actually not necessary to repeat results that are presented in the table in the text again.
8. An interesting observation to the reviewer is the very high rate of rifampicin mono/poly resistance – if the MDR-TB numbers are correct, 15 (30%) of these children had rifampicin mono/poly resistance. Do the authors have an explanation for this? This also has implications both for treatment and preventive therapy in contacts, as INH should still be effective. It would also be interesting to know how many of the MDR-TB cases had additional resistance to ofloxacin (PreXDR-TB)
9. Discussion: As mentioned above, this study cannot be used to determine DR-TB in the children of Pakistan or even in this setting, as the study method of highly selected children (or rather, adolescents) does not at all represent the TB population. This data cannot be compared to data of more general drug resistance surveys. This should be mentioned as study limitations. What it does show is that in a carefully selected high risk group of adolescents not responding well to TB treatment after two months, the rate of DR-TB is very high. However, it would be far more appropriate to do DST on all children with bacteriologically confirmed TB before starting any TB treatment.

Minor comments:

1. Abstract:
 - Line 2: suggest changing “rapidly” to “increasingly”.
 - Line 3: regarding definition - add: (*Mycobacterium tuberculosis* resistant to...).
 - Line 6: suggest: “...smear positive for acid-fast bacilli after...”.
 - Line 8: suggest: “drug susceptibility testing”.
 - Line 12 (Results): Start with total number of children in study: “Fifty children, 32 male (64%) ... were included.
2. Methods:
 - Page 3, 2nd column, line 16: suggest change “not susceptible” to “resistant”.

- Further, the authors use both “sensitive/sensitivity” and “susceptible/susceptibility” in the manuscript – for consistency suggest change all to “susceptible/susceptibility”.
- 3. Results:
 - Table 1: Do the authors know the concordance of child and adult source cases’ *M. tuberculosis* strains DST results in the source case/child contact pairs?
- 4. Discussion:
 - Page 4, 1st column, line 5: suggest: “..., along with child household contacts of MDR-TB cases not being screened and managed appropriately,...”
 - Page 4, 1st column, line 9: not RIF mono-resistant but resistant to RIF.
 - Page 4, 1st column, line 10: extensive drug resistance.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Childhood tuberculosis with specific interest in drug-resistant tuberculosis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 11 Apr 2019

Uzair Yaqoob, Jinnah Postgraduate Medical Centre, Karachi, Pakistan

Thank you so much for this great and comprehensive review, we will surely consider all comments and upload the updated versions with editing done as much as we can.

Competing Interests: No competing interests were disclosed.

Author Response 17 Apr 2019

Uzair Yaqoob, Jinnah Postgraduate Medical Centre, Karachi, Pakistan

Response to comments is following, a newer version has been uploaded. the reviewer will soon see the newer version.

Major comments

1. Done
2. Done
3. Done
4. Rightly said, non-compliant has been defined
5. We were only allowed to access patients and data at the time of the study and currently we cannot access hospital data.
6. Done
7. Done
8. Done

Minor comments:

1. Done
2. Done, sensitive/sensitivity looked more appropriate esp in tables
3. Authors are not aware of this
4. Done

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research