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ORIGINAL RESEARCH

The effect of first-line TB treatment on carbapenem-resistance in faecal *Enterobacterales*

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Carbapenems are used more regularly as empiric and targeted treatment options due to the emergence of resistant bacteria. There is an increased risk of mortality and treatment cost in cases where carbapenem-resistant *Enterobacterales* (CRE) have been isolated. This is due to the fact that the remaining therapeutic options are limited, potentially toxic, and expensive. Antibiotic use is a risk factor for colonisation with resistant bacteria such as CRE and carbapenemase-producing *Enterobacterales* (CPE). Tuberculosis (TB) treatment specifically has not been evaluated as a risk factor for CRE colonisation in the literature. This study aimed to determine whether patients receiving TB treatment are more likely to be colonised with CRE two weeks after treatment is commenced, by collecting a rectal swab before treatment commences, and again two weeks after first-line treatment started. Each collected swab was screened using culture CARBA-R screening plates and the results were evaluated.

Keywords: *Enterobacterales*, carbapenem-resistance, TB treatment

Background

Carbapenems are beta-lactam antibiotics belonging to three out of the four Ambler classification classes for beta-lactamases (Class A, Class B, and Class D) and can be used to treat a variety of infections, such as urinary tract infections, pneumonia, gynaecological infections, and soft tissue infections. The carbapenems are indicated for antimicrobial therapy in cases where resistance to first-line agents is expected, as they have a broad spectrum of activity. The carbapenems are active against many Gram-positive and anaerobic organisms, but their main benefit has been a broader activity against resistant Gram-negative bacteria, especially extended-spectrum beta-lactamases (ESBLs).[1]

There is an increased risk of mortality and treatment costs in cases where carbapenem-resistant *Enterobacterales* (CRE) have been isolated. This is because the remaining therapeutic options are limited, potentially toxic, and expensive.[2,3] Resistance to carbapenems can be intrinsic, i.e. due to chromosomal carbapenemases, efflux pumps or reduction in outer membrane permeability due to porin loss or plasmid-mediated, which is an acquired resistance mechanism. Resistance can also occur due to a combination of ESBL or AmpC beta-lactamase and a deficiency of porins in the outer membrane, namely ESBL and impermeability or AmpC and impermeability. The CRE organisms that are resistant to carbapenems due to the production of carbapenemases are known as carbapenemase-producing *Enterobacterales* (CPE). The resistance to carbapenems in CPE is largely plasmid-mediated and thus is acquired. Carbapenemase production is the most common resistance mechanism.[4] The incidence of CPE has been increasing in South Africa over recent years.[5]

Risk factors for acquiring CRE include exposure of patients to high-care hospital settings, long-term care facilities (LTCF), invasive procedures or foreign devices, and contact with a CRE patient. Patients who have comorbidities and antibiotic exposure are also at risk and thus community-associated cases have been described.[3,6] This increased likelihood of exposure is due to numerous human medical advancements in surgery, neonatal care, organ and tissue transplants, as well as haemato-oncology over the years. These advancements have led to increased length of hospitalisation and exposure to prolonged antimicrobial therapy as well as other antimicrobial agents, which all may affect the composition of the human gut flora and consequently increase the risk of being colonised with a multi-drug resistant organism such as CRE.[7]

Exposure to any agent that has antimicrobial effects, including but not limited to antibiotics/antifungals/chemotherapeutic agents, can affect the composition of gastrointestinal bacteria and result in dysbiosis. Treatment with any antibiotic class regardless of duration has been shown to result in dysbiosis.[7,8] As the microbiome composition is rapidly altered by exposure to antibiotics, the environment becomes more conducive to colonisation with opportunistic pathogens.[8] Antimicrobial therapy affects the intestinal bacteria and can lead to horizontal transfer and selection of resistance due to selection pressure. Gut bacteria readily exchange antibiotic-resistance genes with other bacteria in the gut. Studies indicate that the dysbiosis returns to

its normal state but may not return to the same initial state after an insult.[9]

Tuberculosis (TB) treatment regimens, such as the first-line TB medications which are antimicrobials, have also been shown to cause a dysbiosis of microbial flora; noted when performing gut microbiome studies.[8,10] Microbial dysbiosis is in turn associated with an increased likelihood of acquisition of organisms containing resistance genes. The mechanism by which this happens is due to the normal balance of the occurring gut flora. When the balance of microbial flora in an ecological niche is disrupted, there is less colonisation resistance. Colonisation resistance is a term that describes the ability of a diverse health population of organisms to prevent colonisation/invasion by other potentially pathogenic organisms. This is because a niche is created when the normal flora is destroyed by an antimicrobial, allowing pathogenic organisms to invade and populate the area. Based on this evidence, there is an expectation to observe a change in carbapenem-sensitivity patterns in *Enterobacterales* pre-treatment and after treatment has commenced due to the dysbiosis caused by the treatment itself. There is a likelihood that increasing carbapenem MIC values may be observed in participants during this project and thus increase the risk of CRE/CPE colonisation in the gut.

In South Africa, the TB epidemic poses a huge risk to the health sector as it may lead to increased morbidity and mortality if not effectively managed.[11,12] South Africa is ranked as one of the 30 high-TB-burdened countries globally by the World Health Organization (WHO), with the Western Cape Province reporting one of the highest TB incidences in the country.[13]

The standard TB regimen prescribed for the treatment of drug-susceptible pulmonary TB is usually administered for at least six months, thus patients are exposed to antimicrobial effects for a prolonged period. This regimen contains a combination of agents: isoniazid (INH), rifampicin, ethambutol, and pyrazinamide (PZA) which are taken daily.

As the TB incidence in South Africa and the Western Cape is high in relation to the rest of the world, there will be a significant proportion of the population on TB treatment, which in turn leads to prolonged exposure of the gut microbiota to combination antibiotics. It is therefore worthwhile investigating the potential impact the TB regimen may have as a potential risk factor for acquiring CRE or colonisation of the gut microbiota during this time.

Methods

For this study, a step-wise diagnostic approach was performed that made use of culture media to screen for CRE.[14] If the screening test was positive, the organism identification and susceptibility were performed on the VITEK® 2 automated system to confirm the presence of a CRE.[15] Molecular gene testing then followed to confirm whether the organism was a CPE.[16] Both the pre-treatment baseline sample and the sample on day 14 of treatment were screened and identified using the same laboratory methods. Any changes in organism susceptibility between the two time points noted were analysed.

Patient rectal swabs were collected from the TASK clinical site in Belville, Cape Town from 1 February 2020 until 30 June 2021. All swabs were analysed at Lancet Laboratories, ISO15189 accredited medical laboratory in Century City, Cape Town. The test kits, media, and reagents used underwent quality control testing on arrival. For the screening test, chromID® CARBA SMART agar, a previously identified carbapenemase-producing *Klebsiella pneumonia spp pneumoniae* strain with NDM and OXA48 was used. The agar plates were only used if they passed the quality control checks.

During this study it was important to assess the risk of CRE colonisation of the participants, other than TB treatment, as the participants may have been exposed to other risk factors for CRE colonisation during the 14-day clinic stay at the TASK site. After signing the informed consent form, each participant completed a questionnaire to assess their exposure to other risk factors (refer to Table I for the questions).

Ethical approval was obtained from the Health and Wellness Sciences Research Ethics Committee, registration number NHREC: REC-230408-014; REC Approval Reference Number: CPUT/HW-REC2019/H31.

Informed consent was obtained for each participant per South African Good Clinical Practice Guidelines. Participant confidentiality was maintained by only including the age, gender, and initials on the request forms.

Results

In total, 18 patients were sampled out of the planned 25. This was due to the COVID-19 pandemic and various governmental restrictions imposed. All patients were given Rifafour as treatment over the 14-day period. The outcomes for the patient questionnaires are in Table I.

In total, 36 rectal swabs were collected from 18 patients. At collection, it was noted that the swabs were visibly soiled with faecal matter and were therefore good quality specimens for laboratory investigations.

There were no positive screening results for either the baseline or particularly the 14-day swabs, and therefore no CRE isolated from the 18 patients sampled for this project.

In the questionnaires obtained from the 18 patients, two were exposed to risk factors prior to receiving first-line TB treatment and being sampled. Patient 2 was hospitalised for three days in November 2019 within four months of being sampled for this project and was on antibiotic treatment for a chest infection before both the TB diagnosis and sampling for this project. Patient 6 received antibiotic treatment for pyrexia within one year of sampling and had an ESBL-producing *E. coli* isolated from the baseline swab which was not isolated at 14 days. In both cases, it is unclear with which antibiotic the patients were treated and the duration of the treatment. The remaining 16 patients did not indicate a positive response to risk factor exposure.

Table I: Patient questionnaire responses

Patient number	Have you been hospitalised in the last year?	Have you been hospitalised in a high-care/ICU ward?	Any contact with ICU/LTCF discharged person?	Any operations/ procedures or catheters inserted in last two years?	Any antibiotics in the last year, e.g., TB Rx?	Any chronic illness: diabetes, asthma, etc.?
1	No	No	No	No	No	No
2	Yes*	No	No	Yes*	Yes*	No
3	No	No	No	No	No	No
4	No	No	No	No	No	No
5	No	No	No	No	No	No
6	No	No	No	No	Yes*	No
7	No	No	No	No	No	No
8	No	No	No	No	No	No
9	No	No	No	No	No	No
10	No	No	No	No	No	No
11	No	No	No	No	No	No
12	No	No	No	No	No	No
13	No	No	No	No	No	No
14	No	No	No	No	No	No
15	No	No	No	No	No	No
16	No	No	No	No	No	No
17	No	No	No	No	No	No
18	No	No	No	No	No	No

Patient 2 was hospitalised for three days in November 2019 within four months of being sampled for this project and was on antibiotic treatment for a chest infection six days before the official TB diagnosis and sampling for this project; **Patient 6** received antibiotic treatment for pyrexia within one year of sampling

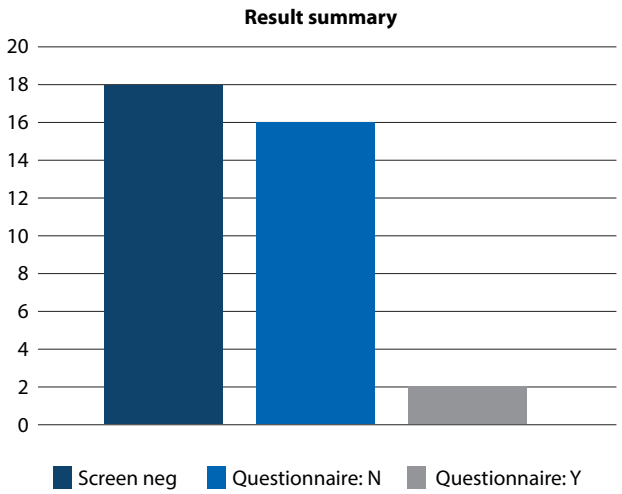
ICU – intensive care unit, LTCF – long-term care facility, TB Rx – tuberculosis treatment

Discussion

From the 18 patients sampled, there was no CRE isolated, all patients' screening tests were negative and there was, therefore, no need for molecular investigations as no significant organisms were isolated (see result summary below).

Although the sampled patients are a small group, the results could indicate the following:

- The sampled patients were not exposed to CPE/CRE during the 14-day period:



A study conducted in mice indicates that the timing of exposure to CPE as well as antibiotic usage may be a key component when considering colonisation.[17] In this study, mice were exposed to CPE and a single dose of clindamycin, an antimicrobial to which *Enterbacterales spp*. are resistant. Single clindamycin injection within a week before or after CPE exposure induced gut colonisation for at least 100 days. Since the first-line TB treatment regimen is a six-month course, our study may indicate that patient screening should extend to at least the end of the treatment and antibiotic exposure.

- It could take longer for patients to be colonised with CRE while on TB treatment:
  A study in 2017 by Wipperman et al. shows that combination TB treatment does have a significant effect on the microbiome and profound dysbiosis was noted one to two years after the combination therapy.[8]
  A limitation of our project is that 18 patients are a small number and since there has been no CRE noted at screening or after 14 days' treatment, it cannot be ruled out that it can occur. The sampled patient group is not large enough to determine this. Furthermore, the two-week period may also not be sufficient to detect changes to the *Enterbacterales spp*. in the gut microbiome.
- It also may take longer to detect CRE by culturing rectal swabs for patients on TB treatment:
  The detection of CRE using culture methods may take a longer period of exposure to first-line treatment to be detected. Of

the 18 patients that were sampled, CRE was not isolated in any patient. For future studies, a larger proportion of the population will have to be sampled and over a treatment period exceeding 14 days; potentially the six-month period for the first-line treatment regimen.

The methods used for the project are sound and aligned with the routine detection methods. A study in 2018 by Knight et al. demonstrated that the use of culture and PCR provides the optimal balance of cost and risk of days averted.[18] As limited funds were available for this study, it was important to balance the cost and sensitivity of results.

- CRE may be generally uncommon in the population on TB treatment:

  By using the patient questionnaire obtained at both time points, we were able to exclude other risk factors.

  From the first-line combination therapy, rifampicin is a broad-spectrum antibiotic which inhibits bacterial RNA polymerase, which is a broad-spectrum antimicrobial that is used for non-mycobacterial infections. Additionally, rifampicin has a broad spectrum of activity, including possible effects on other organisms in the gut microbiota with some Gram-positives and Gram-negatives in combination or alone.
- The first-line treatment prodrugs are PZA and INH, which are activated only within the mycobacterium. PZA is activated to Pyrazinoic acid in the bacilli where it interferes with fatty acid synthase and interferes with the organism's ability to synthesise new fatty acids required for growth and replication. INH is activated by bacterial catalase, and once activated, isoniazid inhibits the synthesis of mycoloic acids, an essential component of the bacterial cell wall. These prodrug properties may have reduced the anticipated effect of the combination therapy on the microbiome and subsequent dysbiosis, as the drug is only active once inside the mycobacterium. These mechanisms could influence the dysbiosis properties of these microbial agents.

  The literature does support significant dysbiosis.[8] However, this project suggests that within the small group of 18 patients sampled, 14 days of treatment may not have been sufficient time to detect the resultant CRE and CPE. The population sampled was community-based and diagnosed with pulmonary TB, but were otherwise healthy.
- CRE carriage in the general population in TB-endemic areas in the world and the duration of time on treatment required to be colonised with CRE:

  Tuberculosis remains a problem worldwide according to the global TB report 2020. TB cases are reported in the WHO regions of South-East Asia (44%), Africa (25%) and the Western Pacific (18%), with smaller shares in the Eastern Mediterranean (8.2%), the Americas (2.9%) and Europe (2.5%).

  Eight countries account for two-thirds of the global total: India (26%), Indonesia (8.5%), China (8.4%), Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%) and South Africa (3.6%).[19] According to the GERMSA report for 2020, the number of CRE bacteraemia cases had steadily increased annually from 2015 to 2019.[20] The literature shows that both TB and CRE remain a concern. To date, it is not clear from the literature whether there is an association and if TB medication is a separate risk factor for colonisation by CPE/CRE. It is therefore difficult to determine the risk of CRE carriage.

Conclusion

At the onset of the study, the plan was to sample 25 patients and evaluate the findings. Due to the COVID-19 pandemic, 18 willing participants consented and were sampled at baseline and after 14 days of TB first-line treatment. Participant rectal swabs were screened at baseline and at 14 days of treatment with the result that zero patients were positive for the screening test. Although no CRE was isolated in these 18 patients, due to the small sample size, the possibility cannot be excluded.

In conclusion, from the 18 patients sampled, no CRE was isolated. However, this may be due to the small sample size and the timing of the sampling. The possibility that patients exposed to first-line TB treatment may over time be colonised with CRE, cannot be excluded.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Ethical approval

Ethical approval was obtained from the CPUT Health and Wellness Sciences Research Ethics Committee (HWS-REC), REC Approval Reference No: CPUT/HW-REC 2019/H31.

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