Tebipenem as an oral alternative for the treatment of typhoid caused by extensively drug resistant (XDR) *Salmonella* Typhi

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Brief Communication

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Abstract

The emergence of multi-drug (MDR) and extensive-drug resistance (XDR) in *Salmonella* Typhi and Paratyphi A hinder efficacious out-patient enteric fever treatment. We show that non-XDR and XDR *S*. Typhi and *S*. Paratyphi A are susceptible to the carbapenem tebipenem *in vitro*. Tebipenem demonstrated partial synergy with antimicrobials including azithromycin, signifying combination therapy may limit the emergence of resistance. Given recent evidence on tebipenem inhibitory activity against MDR *Shigella*, its broad-spectrum activity against MDR/XDR organisms renders it a good clinical trial candidate.

Main

Antimicrobial resistance (AMR) poses a major threat for enteric fever treatment, as well as infections caused by other Gram-negative bacteria, such as *Shigella* spp. and pathogenic *Escherichia coli* [1]. Enteric (typhoid) fever is a life-threatening systemic disease caused by *Salmonella enterica* serovar Typhi (*S*. Typhi), and the various pathovars of *S*. Paratyphi (A, B and C). Enteric fever remains a public health problem in many countries in South Asia and sub-Saharan Africa with poor sanitation, resulting in an estimated global incidence of >14 million cases and >135,000 deaths annually [2].

Multi-drug resistant (MDR) *S.* Typhi (resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole) has become common and been facilitated by the global expansion of the H58 lineage [3]. More recently, extensive-drug resistant (XDR) *S.* Typhi, characterised by resistance to fluoroquinolones and third generation cephalosporins in combination with the standard MDR phenotype have been isolated in Pakistan [4]. XDR *S.* Typhi have since been identified in other countries and been associated with travel to Pakistan [5,6]. Alarmingly, cases of XDR typhoid (identical susceptibility profile as isolates in Pakistan) with no recent travel history have been recently recorded in USA [7].

XDR *S*. Typhi isolates remain largely susceptible to azithromycin and carbapenems [4,8], with guidelines in Pakistan and the American CDC recommending these antimicrobials in monotherapy or in combination for the treatment of XDR typhoid infections [7]. However, azithromycin resistance has been recorded in both *S.* Typhi and *S.* Paratyphi A and appears to be increasing [9,10]. The carbapenems are a potent class of beta-lactam antimicrobials used to treat life-threatening bacterial infections and XDR typhoid can be effectively treated by meropenem or imipenem. Unfortunately, these antimicrobials are administered parenterally, thus restricting their use largely to inpatients.

Our repertoire of oral antimicrobials against MDR/XDR organisms is becoming limited and the emergence of XDR *S*. Typhi highlights the need for alternative antimicrobials to treat infections associated with these highly resistant organisms. We recently identified tebipenem as repurposing opportunities for infections caused by MDR *Shigella,* with clinical *Shigella* isolates exhibiting MIC values of 0.04 to 0.3 μM [11]. The prodrug, tebipenem pivoxil, is an oral carbapenem that is only licenced for use in paediatric patients with serious respiratory infections in Japan [12]. It presents with high oral bioavailability, broad spectrum, and activation in gut enterocytes potentially offers a solution for treating

XDR infections without the requirement for hospitalisation and Spero Therapeutics are developing an adult formulation with an extended-half life [11]. The reported breakpoints for tebipenem propose that tebipenem-susceptible bacteria have MIC values <1 μg/mL[12].

To determine the tebipenem repurposing potential for typhoidal *Salmonella*, we measured the inhibitory activity of this compound against a collection of 100 clinical non-XDR and XDR *S.* Typhi and non-XDR *S.* Paratyphi A from Pakistan and Nepal. The MIC values for tebipenem against tested isolates were consistently ≤0.62 μg/mL (or 1.25 μM) (IQR: 0.12–0.25 μg/mL or 0.24–0.5 μM; Figure 1A), even in the XDR isolates. The majority of *S.* Typhi from both Pakistan (XDR) and Nepal (non-XDR) had lower MIC values (median of 0.12 μg/mL and 0.039 μg/mL, respectively) compared to Nepali *S.* Paratyphi A (non-XDR) (median of 0.31 μg/mL); the latter also included the least tebipenem-susceptible isolates in our collection (ED199, 02TY067, DM188, and ED293 with a MIC of 0.62 μg/mL). These data suggest that the drug is likely to work in enteric fever patients infected with XDR and non-XDR isolates.

Identifying that all organisms were susceptible to tebipenem we selected two isolates (*S.* Typhi 01TY257 and *S.* Paratyphi A 02TY224), to further investigate the bactericidal effect of tebipenem against typhoidal *Salmonella*. The tebipenem MIC and minimum bactericidal concentration (MBC) values of *S.* Paratyphi A 02TY224 was 4 and 8 times higher, respectively, compared to these of *S.* Typhi 01TY257 (Figure 1B). Time-kill assays of tebipenem showed that the compound exhibited high level bactericidal activity against both isolates, with rapid killing occurring during the first 6 hours of exposure (Figure 1C-D). *S.* Typhi 01TY257 was effectively killed by tebipenem at 2XMIC after 24 hours and at 4XMIC within 8 hours (Figure 1C). In comparison, tebipenem induced complete killing of *S.* Paratyphi A 02TY224 at 4XMIC only after 24 hours of exposure (Figure 1D). Notably, both *S.* Typhi and *S.* Paratyphi A recovered growth when treated with 0.5–1XMIC after 6 to 8 hours of tebipenem exposure (Figure 1C-D).

Carbapenems remain the last resort treatment for many infections and thus the emergence of resistance has to be mitigated. However, carbapenem resistance is not uncommon [13], and many bacterial pathogens causing nosocomial infections, such as *Klebsiella pneumoniae*, employ resistance mechanisms such as plasmid-borne carbapenemases and/or modify outer membrane influx proteins [14,15]. Combining tebipenem with other commonly used antimicrobials with different modes of action may prove to be effective in reducing the risk of developing resistance to carbapenems [11]. We determined the synergistic abilities of tebipenem combined with azithromycin and an LpxC inhibitor (PF-5081090) in *in vitro* assays. Tebipenem combined with either the LpxC inhibitor or azithromycin resulted in partial synergy (FICI scores of ≤0.5) for both tested *S.* Typhi and *S.* Paratyphi A isolates (Figure 1E), and notably even for azithromycin-resistant *S.* Paratyphi A 02TY224 (Table S1). These results suggest that these combinations may be beneficial to protect the efficiency of tebipenem and limit the emergence of resistance to this vital class of antimicrbials. Given the high *in vitro* potency of tebipenem against a range of enteric pathogens and the prodrug hydrolysis and active ingredient release within enterocytes, we suggest it could be administered before obtaining culture results when XDR typhoid is considered [11].Tebipenem is already licensed in Japan (Orapenem) to treat paediatric respiratory infections and has

an existing safety documentation [12], rendering it an attractive compound for clinical trials in MDR/XDR typhoidal *Salmonella*.

We are in urgent need of new antimicrobials for the treatment of infections caused by XDR organisms and the emergence of XDR typhoid in Pakistan and the USA has left azithromycin as the only remaining oral alternative. Our data show that Orapenem (tebipenem pivoxil) may offer some respite in the community treatment of XDR enteric fever and that resistance may be prevented by combining this carbapenem with an antimicrobial with an alternative mode of action.

Methods

The *S.* Typhi and *S.* Paratyphi A organisms used in this study were previously isolated in Nepal (n=21; non-MDR/non-XDR) [16,17] and in Pakistan (n=79 organisms, all XDR). Bacteria were cultured in Mueller Hinton (MH) (Sigma Aldrich, UK) media overnight at 37 °C. The minimum inhibitory concentrations (MIC) values for tebipenem (Sigma Aldrich, UK) were determined by an existing micro-dilution assay [11]. Briefly, 10μM tebipenem in MH broth was serially diluted and 5×10^5 CFU/ml bacteria were added and incubated at 37^{o}C overnight. Bacterial growth was detected by plating 10μl of each well on Nutrient Agar (NA, Oxoid) and incubating overnight at 37^{o}C. Results were interpreted as the minimal concentration necessary to inhibit growth.

Time-kill curve assays were performed in 50ml falcon tubes by culturing *Salmonella* in MH medium in the presence of four antimicrobial concentrations in doubling dilutions ranging from 0.5xMIC to 4xMIC. Bacterial stocks were prepared in 0.9% NaCl and added into each tube to obtain a concentration of 5×10^5 CFU/mL. Bacteria were grown with agitation at 200 rpm at 37^{o}C and monitored over a time-course of 24 hours (0, 2, 4, 6, 8, and 24 hours). For every concentration and time point, bacterial cultures were diluted and inoculated onto NA (Oxoid), before being incubated at 37^{o}C overnight and CFUs were enumerated.

Combination studies with clinical isolates were performed as previously described [11].MICs were determined for drug A and drug B alone and in combination. The MIC of both drugs in combination were expressed as fractions of the MIC of the drug alone normalized to 1, which represents the Fractional Inhibitory Concentration (FIC). The sum of FIC was expressed using the following equation: (MIC of drug A in combination / MIC of drug A alone) + (MIC of drug B in combination / MIC of drug B alone) giving the FIC index (FICI) score.

Declarations

Ethics approval and consent to participate

Not required

Consent for publication

Not required

Availability of data and materials

All data are presented in the manuscript and raw data are freely available upon request from the corresponding author.

Competing interests

The authors declare no competing interests.

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Table

Table S1. Antimicrobial profile of typhoidal *Salmonella* isolates from Nepal

Organism	Nalidixic acid	Ciprofloxacin	Ceftriaxone	Ceftazidime	Amoxicillin-clavulanate	Ampicillin	Trimethoprim-	Azithromycin	Chloramphenicol	Imipenem	Amikacin	Gentamycin	ESBL
SPA 02TY067	R	I	S	S	S	S	S	S	S	S	S	S	NEG
SPA 02TY224	R	S	S	S	S	S	S	R	S	S	S	S	NEG
SPA 02TY242	R	I	S	S	S	S	S	R	S	S	S	S	NEG
SPA DM188	R	S	S	S	S	S	S	R	S	S	S	S	NEG
SPA ED293	R	I	S	S	S	S	S	R	S	S	S	S	NEG
STY 01TY257	S	S	S	S	S	S	S	S	S	S	S	S	NEG
STY 01TY258	S	S	S	S	S	S	S	S	S	S	S	S	NEG
STY 01TY151	S	S	S	S	S	S	S	S	S	S	S	S	NEG
STY 01TY154	S	S	S	S	S	S	S	S	S	S	S	S	NEG
STY 01TY086	S	S	S	S	S	S	S	S	S	S	S	S	NEG

Figures

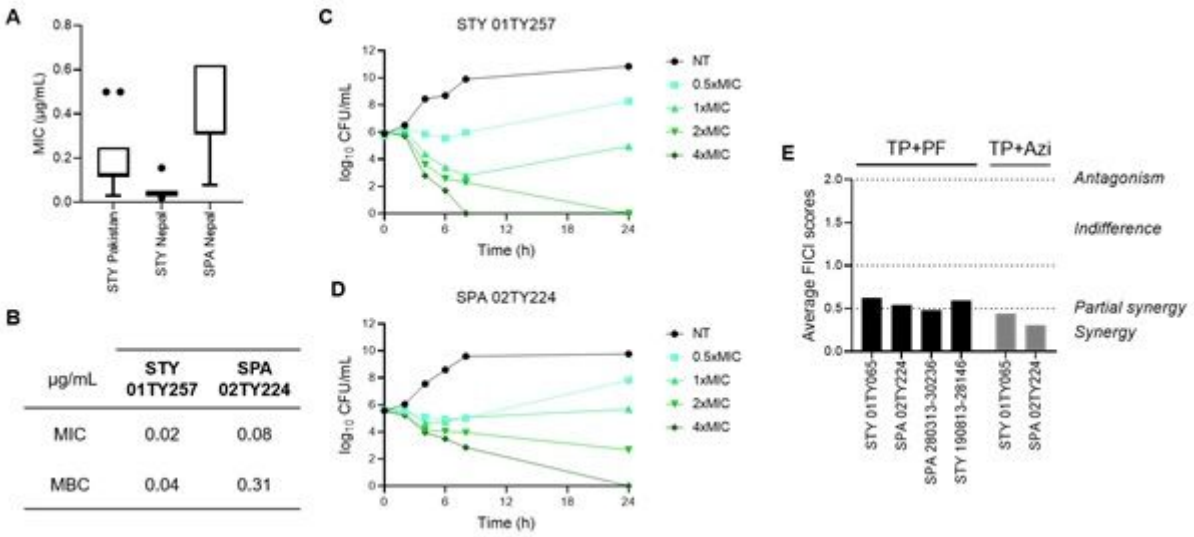

Figure 1

Tebipenem as an oral alternative for the treatment of enteric fever caused by MDR/XDR S. Typhi/S. Paratyphi A (A) Tukey boxplots showing MIC values of S. Typhi (STY) and S. Paratyphi A (SPA) isolated from Pakistan and Nepal against tebipenem. The isolates from Pakistan are all XDR S. Typhi. (B) Tebipenem MIC and MBC values of STY 01TY257 and SPA 02TY224. (C-D) Representative time-kill curves of STY (C) and SPA (D) isolates in various doubling concentrations of tebipenem compared to bacteria grown with no treatment (NT). (E) Bar chart showing the average FICI scores to determine the in vitro synergy or antagonism of tebipenem (TP) in combination with an LpxC inhibitor (PF; black) or Azithromycin (Azi; grey) against various STY or SPA isolates.