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Activity of propyl-propane-thiosulfinate and propyl-propane-thiosulfonate against carbapenem-resistant Gram-negative bacteria

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Organosulfur compounds derived from plants of the *Allium* genus, such as propyl-propane-thiosulfinate (PTS) and propyl-propane-thiosulfonate (PTSO), have been proposed as an alternative in antibiotic resistance. The aim of this study was to compare the activity of these substances with other antibiotics against clinical isolates of carbapenem-resistant (CAR-R) and carbapenem-susceptible (CAR-S) Gram-negative bacteria. A total of 126 clinical isolates of CAR-R and 155 CAR-S bacteria were selected, including Enterobacterales, *A. baumannii* and *P. aeruginosa*. The antibiotic susceptibility of all isolates was assessed using the microdilution and Kirby–Bauer methods for PTS, PTSO, amoxicillin/clavulanate, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, imipenem, ciprofloxacin, and amikacin. Both PTS and PTSO demonstrated *in vitro* bactericidal activity against CAR-R *Enterobacteriaceae* and *A. baumannii*, with no significant difference in activity compared to their response against CAR-S isolates. However, both compounds were less active against *P. aeruginosa* than against any of the other bacteria, regardless of their resistance to carbapenems. In all cases, the minimum inhibitory concentration values of PTSO were significantly lower than those of PTS. These findings offer valuable information about the potential antibacterial use of these substances, particularly against infections that currently have limited therapeutic options.

Key words: Gram-negative bacilli; carbapenemases; propyl-propane-thiosulfinate; propyl-propane-thiosulfonate; antibacterial activity.

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Bacterial resistance to antibiotics poses a significant threat to human health and requires urgent attention. This resistance has greatly reduced the effectiveness of treatments for infectious diseases caused by bacteria [1]. Carbapenem-resistant (CAR-R) Enterobacterales, including *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter* spp., as well as *Pseudomonas aeruginosa* or *Acinetobacter baumannii*, are often resistant to other antibiotic groups, such as aminoglycosides and fluoroquinolones. These bacteria are responsible for increased hospital admissions, higher sanitary costs and a high percentage of lethality in seriously ill or immunocompromised patients [2, 3]. Although their presence in our territory (Granada, Spain) is low [4], their global incidence represents a clinical threat and is becoming increasingly concerning in some European countries [5, 6]. Therefore, it is necessary to establish appropriate measures to prevent an increased prevalence [7].

New strategies have emerged to address the reduction of therapeutic options for the treatment of infectious diseases. One such strategy involves studying the antibacterial properties of molecules

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Table 1. Mechanisms of carbapenem resistance detected in the 126 clinical isolates.

Mechanism of resistance	*K. pneumoniae*	*E. coli*	*E. cloacae*	*C. freundii*	*A. baumannii*	*P. aeruginosa*	Total
Lack of OprD						1	1
IMP						3	3
KPC		1		2			3
VIM			11			5	16
OXA-23					40		40
OXA-48	39	8	10	1			58
KPC + VIM	1						1
KPC + OXA-48	1						1
VIM + OXA-48				2			2
NDM + OXA-48	1						1

IMP, active-on-imipenem carbapenemase, KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-betalactamase; OXA, OXA-type carbapenemase; VIM, Verona integron-encoded metallo-betalactamase.

derived from plants [8]. Throughout history, various civilizations have used different plant species in traditional medicine due to their accessibility and lack of side effects. Plant extracts are still being used to develop new drugs, as they contain metabolites with a broad spectrum of activity [9]. Some of these products have been shown to be effective in treating microorganisms that are resistant to commonly used antibiotics in clinical practice. This has led to the exploration of new active treatments [10].

In this context, plants of the *Allium* genus produce a range of organosulfur compounds that exhibit antibacterial, antifungal, antiviral, and antiparasitic properties [11, 12]. *Allium cepa* (onion) produces methiin (S-methyl-L-cysteine sulfoxide), isoaliin (S-propenyl-L-cysteine sulfoxide), and propiin (S-propyl-L-cysteine sulfoxide). Propiin undergoes changes due to the action of alliinase, forming propyl-propane-thiosulfinate (PTS), which is then transformed into dipropyl disulfide and propyl-propane-thiosulfonate (PTSO) through dismutation or disproportion reactions [13]. Previous studies have compared the antibacterial activity of PTS and PTSO with that of other antibiotics, demonstrating significant broad-spectrum antibacterial activity against a variety of clinical isolates of Gram-positive and Gram-negative bacteria [14, 15].

The aim of this study was to compare the antibacterial activity of PTS and PTSO with other antibiotics against a selection of CAR-R and carbapenem-susceptible (CAR-S) Gram-negative bacteria *in vitro*.

RESULTS

Table 1 displays the mechanisms of resistance to carbapenem antibiotics identified in the 126 CAR-R isolates. In 125 of these, resistance was caused by the presence of one or more carbapenemases. Of these, 81.6% expressed an OXA-type carbapenemase, either alone (78.4%) or in combination with another carbapenemase. The study found that the OXA-48 carbapenemase was the most prevalent enzyme, present in 49.2% of the CAR-R isolates, exclusively in *Enterobacteriaceae*. The second most common enzyme detected was OXA-23 (31.7%), which was exclusively found in *A. baumannii*.

Table 2 provides a summary of the MIC_{50}, MIC_{90}, MBC_{50}, MBC_{90}, and resistance percentages of the antibacterial agents tested by microdilution of the 126 CAR-R and the 155 CAR-S clinical isolates. CAR-S isolates exhibited resistance to amoxicillin/clavulanate (100% of *E. cloacae*, *C. freundii*, *A. baumannii*, and *P. aeruginosa*; 40% of *E. coli* and 25% of *K. pneumoniae*), piperacillin/tazobactam (100% of *A. baumannii*, 40% of *P. aeruginosa*, 16.7% of *K. pneumoniae*, and 10% of *E. coli*), and cefotaxime (100% of *A. baumannii* and *P. aeruginosa*). However, susceptibility to all other beta-lactam antibiotics was observed.

One relevant characteristic of the 126 CAR-R isolates was their high frequency of co-resistance to other non-beta-lactam antibiotics that were tested. These bacteria demonstrated resistance to ciprofloxacin, with rates of 100% for *A. baumannii*, 97.6% for *K. pneumoniae*, 85.7% for *E. cloacae*, 80% for *C. freundii*, 55.6% for *E. coli*, and 44.4% for *P. aeruginosa*. They also showed resistance to amikacin, with rates of 95% for *A. baumannii*, 22.2% for *P. aeruginosa*, 16.7% for *K. pneumoniae*, and 4.8% for *E. cloacae*. It is important to note that 36.5% of CAR-R isolates were not only resistant to beta-lactams but also to ciprofloxacin and amikacin, making them multidrug-resistant bacteria (MDR). In contrast, all CAR-S isolates were susceptible to these two antibiotics.

Table 3 presents the results of the Mann–Whitney *U*-test comparing the MIC values of PTS and PTSO among different groups. No significant differences were found in the activity of PTS ($p = 0.881$) and

Table 2. *In vitro* activity of PTS, PTSO, and other antibacterial agents against carbapenem-resistant (CAR-R) and carbapenem-susceptible (CAR-S) bacteria

Bacterial species/antibacterial agent	MIC_{50} (in mg/L)		MIC_{90} (in mg/L)		MBC_{50} (in mg/L)		MBC_{90} (in mg/L)		Percentage of resistant isolates[1]	
	CAR-R	CAR-S	CAR-R	CAR-S	CAR-R	CAR-S	CAR-R	CAR-S	CAR-R	CAR-S
Klebsiella pneumoniae										
Amoxicillin/clavulanate	2048/1024	4/2	>2048/1024	16/8	>2048/1024	32/16	>2048/1024	128/64	100.0	25.0
Piperacillin/tazobactam	2048/4	≤4/4	>4096/4	32/4	>4096/4	32/4	>4096/4	256/4	100.0	16.7
Cefotaxime	>512	≤0.5	>512	≤0.5	>512	2	>512	>4	100.0	0.0
Ceftazidime	128	≤2	512	≤2	1024	16	>2048	>16	100.0	0.0
Cefepime	1024	≤1	>1024	≤1	>1024	4	>1024	>8	100.0	0.0
Imipenem	16	≤0.25	256	0.5	64	0.5	>256	>2	100.0	0.0
Ciprofloxacin	>128	≤0.125	>128	≤0.125	>128	≤0.125	>128	>1	97.6	0.0
Amikacin	≤2	≤2	>2048	8	16	4	>2048	16	16.7	0.0
PTS	128	128	256	256	256	256	512	512	-	-
PTSO	128	128	256	128	128	128	256	256	-	-
Escherichia coli										
Amoxicillin/clavulanate	1024/512	8/4	>2048/1024	32/16	2048/1024	32/16	>2048/1024	128/64	100.0	40.0
Piperacillin/tazobactam	256/4	≤4/4	4096/4	8/4	>4096/4	8/4	>4096/4	64/4	100.0	10.0
Cefotaxime	>512	≤0.5	>512	≤0.5	>512	≤0.5	>512	2	100.0	0.0
Ceftazidime	32	≤2	1024	≤2	256	≤2	>2048	4	100.0	0.0
Cefepime	128	≤1	>1024	≤1	1024	≤1	>1024	4	100.0	0.0
Imipenem	32	≤0.25	256	≤0.25	64	≤0.25	>256	2	100.0	0.0
Ciprofloxacin	32	≤0.125	>128	≤0.125	>128	≤0.125	>128	1	55.6	0.0
Amikacin	4	≤2	16	≤2	8	≤2	128	4	0.0	0.0
PTS	64	64	128	128	256	128	512	256	-	-
PTSO	64	64	128	64	128	64	256	256	-	-
Enterobacter cloacae										
Amoxicillin/clavulanate	1024/512	32/16	>2048/1024	128/64	>2048/1024	512/256	>2048/1024	512/256	100.0	100.0
Piperacillin/tazobactam	1024/4	≤4/4	2048/4	8/4	4096/4	8/4	>4096/4	64/4	100.0	0.0
Cefotaxime	256	≤0.5	>512	≤0.5	>512	2	>512	>4	100.0	0.0
Ceftazidime	256	≤2	512	≤2	2048	16	>2048	>16	100.0	0.0
Cefepime	64	≤1	>1024	2	512	8	>1024	16	100.0	0.0
Imipenem	32	0.5	128	1	128	2	>256	4	100.0	0.0
Ciprofloxacin	128	≤0.125	>128	0.25	>128	0.5	>128	2	85.7	0.0
Amikacin	≤2	≤2	8	≤2	16	8	64	16	4.8	0.0
PTS	128	64	256	256	128	128	256	256	-	-
PTSO	64	64	128	128	128	128	256	256	-	-
Citrobacter freundii										
Amoxicillin/clavulanate	2048/1024	64/32	>2048/1024	128/64	>2048/1024	128/64	>2048/1024	512/256	100.0	100.0
Piperacillin/tazobactam	1024/4	≤4/4	2048/4	≤4/4	>4096/4	32/4	>4096/4	32/4	100.0	0.0
Cefotaxime	256	≤0.5	>512	≤0.5	>512	2	>512	4	100.0	0.0
Ceftazidime	256	4	512	4	1024	8	>2048	16	100.0	0.0
Cefepime	256	≤1	1024	≤1	1024	2	>1024	4	100.0	0.0
Imipenem	16	≤0.25	256	≤0.25	128	1	256	1	100.0	0.0
Ciprofloxacin	128	≤0.125	>128	0.25	>128	≤0.125	>128	1	80.0	0.0

Table 2 *(continued)*

Bacterial species/antibacterial agent	MIC_{50} (in mg/L)		MIC_{90} (in mg/L)		MBC_{50} (in mg/L)		MBC_{90} (in mg/L)		Percentage of resistant isolates[1]	
	CAR-R	CAR-S	CAR-R	CAR-S	CAR-R	CAR-S	CAR-R	CAR-S	CAR-R	CAR-S
Amikacin	≤2	≤2	16	8	16	≤2	128	16	0.0	0.0
PTS	128	64	256	128	256	64	512	128	-	-
PTSO	64	32	128	64	128	64	128	128	-	-
Acinetobacter baumannii										
Amoxicillin/clavulanate	>2048/1024	128/64	>2048/1024	512/256	>2048/1024	512/256	>2048/1024	1024/512	100.0	100.0
Piperacillin/tazobactam	256/4	64/4	2048/4	128/4	>4096/4	256/4	>4096/4	512/4	100.0	100.0
Cefotaxime	512	64	>512	128	512	128	>512	256	100.0	100.0
Ceftazidime	128	≤2	512	≤2	512	4	1024	16	100.0	0.0
Cefepime	64	2	256	4	256	16	>1024	32	100.0	0.0
Imipenem	>256	≤0.25	>256	≤0.25	>256	2	>256	2	100.0	0.0
Ciprofloxacin	128	≤0.125	>128	0.25	>128	0.5	>128	1	100.0	0.0
Amikacin	>2048	4	>2048	8	>2048	8	>2048	16	95.0	0.0
PTS	64	128	128	256	128	256	512	512	-	-
PTSO	32	64	64	128	64	64	256	256	-	-
Pseudomonas aeruginosa										
Amoxicillin/clavulanate	>2048/1024	>2048/1024	>2048/1024	>2048/1024	>2048/1024	>2048/1024	>2048/1024	>2048/1024	100.0	100.0
Piperacillin/tazobactam	128/4	≤4/4	4096/4	128/4	512/4	128/4	>4096/4	256/4	100.0	40.0
Cefotaxime	512	16	>512	64	>512	128	>512	512	100.0	100.0
Ceftazidime	512	≤2	>2048	4	512	>16	>2048	>16	100.0	0.0
Cefepime	256	≤1	>1024	4	256	16	>1024	>16	100.0	0.0
Imipenem	>256	1	>256	2	>256	4	>256	8	100.0	0.0
Ciprofloxacin	0.25	≤0.125	>128	0.5	2	1	>128	>1	44.4	0.0
Amikacin	8	≤2	128	8	16	16	128	16	22.2	0.0
PTS	1024	1024	2048	4096	1024	1024	2048	4096	-	-
PTSO	256	512	1024	1024	512	1024	1024	2048	-	-

[1]For each antibiotic, intermediate and resistant organisms (considered according to the interpretive categories and MIC breakpoints of the CLSI) are grouped together.

Table 3. Comparison of MIC values of PTS and PTSO by Mann–Whitney *U*-test

Comparison of PTS activity between CAR-R and CAR-S bacteria	p-Value
There were no significant differences in the MIC values of PTS between the 126 CAR-R bacteria and the 155 CAR-S bacteria	0.881
There were no significant differences in the MIC values of PTS between 42 *K. pneumoniae* CAR-R and 60 *K. pneumoniae* CAR-S	0.106
There were no significant differences in the MIC values of PTS between 9 *E. coli* CAR-R and 50 *E. coli* CAR-S	0.928[1]
There were no significant differences in the MIC values of PTS between 21 *E. cloacae* CAR-R and 10 *E. cloacae* CAR-S	0.582
There were no significant differences in the MIC values of PTS between 5 *C. freundii* CAR-R and 5 *C. freundii* CAR-S	0.211[1]
There were no significant differences in the MIC values of PTS between 40 *A. baumannii* CAR-R and 5 *A. baumannii* CAR-S	0.165[1]
There were no significant differences in the MIC values of PTS between 9 *P. aeruginosa* CAR-R and 25 *P. aeruginosa* CAR-S	0.936
Comparison of PTSO activity between CAR-R and CAR-S bacteria	**p-Value**
There were no significant differences in the MIC values of PTSO between the 126 CAR-R bacteria and the 155 CAR-S bacteria	0.150
There were no significant differences in the MIC values of PTSO in 42 *K. pneumoniae* CAR-R and 60 *K. pneumoniae* CAR-S	0.589
There were no significant differences in the MIC values of PTSO between 9 *E. coli* CAR-R and 50 *E. coli* CAR-S	0.928[1]
There were no significant differences in the MIC values of PTSO between 21 *E. cloacae* CAR-R and 10 *E. cloacae* CAR-S	0.542
There were no significant differences in the MIC values of PTSO between 5 *C. freundii* CAR-R and 5 *C. freundii* CAR-S	0.250[1]
There were no significant differences in the MIC values of PTSO between 40 *A. baumannii* CAR-R and 5 *A. baumannii* CAR-S	0.116[1]
There were no significant differences in the MIC values of PTSO between 9 *P. aeruginosa* CAR-R and 25 *P. aeruginosa* CAR-S	0.110
Comparison of the activity of PTS and PTSO	**p-Value**
The MIC values of PTSO in 281 bacteria were significantly lower than those of PTS	<0.001
The MIC values of PTSO in 126 CAR-R bacteria were significantly lower than those of PTS	<0.001
The MIC values of PTSO in 155 CAR-S bacteria were significantly lower than those of PTS	<0.001
The MIC values of PTSO in 102 *K. pneumoniae* were significantly lower than those of PTS	<0.001
The MIC values of PTSO in 59 *E. coli* were significantly lower than those of PTS	0.004
The MIC values of PTSO in 31 *E. cloacae* were significantly lower than those of PTS	0.018
The MIC values of PTSO in 10 *C. freundii* were significantly lower than those of PTS	0.039
The MIC values of PTSO in 45 *A. baumannii* were significantly lower than those of PTS	<0.001
The MIC values of PTSO in 34 *P. aeruginosa* were significantly lower than those of PTS	<0.001
Comparison of the activity of PTS between different bacterial groups	**p-Value**
The MIC values of PTS in 102 *K. pneumoniae* were significantly lower than those of the remaining 179 bacteria	<0.001
The MIC values of PTS in 42 *K. pneumoniae* CAR-R were significantly lower than those of the remaining 84 CAR-R bacteria	<0.001
The MIC values of PTS in 60 *K. pneumoniae* CAR-S were significantly lower than those of the remaining 95 CAR-S bacteria	0.009
The MIC values of PTS in 59 *E. coli* were significantly lower than those of the remaining 222 bacteria	<0.001
The MIC values of PTS in 9 *E. coli* CAR-R were significantly lower than those of the remaining 117 CAR-R bacteria	0.029[1]
The MIC values of PTS in 50 *E. coli* CAR-S were significantly lower than those of the remaining 105 CAR-S bacteria	<0.001
The MIC values of PTS in 31 *E. cloacae* were not significantly different from those of the remaining 250 bacteria	0.764
The MIC values of PTS in 21 *E. cloacae* CAR-R were not significantly different from that of the remaining 105 CAR-R bacteria	0.976
The MIC values of PTS in 10 *E. cloacae* CAR-S were not significantly different from that of the remaining 145 CAR-S bacteria	0.522
The MIC values of PTS in 10 *C. freundii* were not significantly different from that of the remaining 271 bacteria	0.107
The MIC values of PTS in 5 *C. freundii* CAR-R were not significantly different from that of the remaining 121 CAR-R bacteria	0.719[1]

Table 3 *(continued)*

Comparison of the activity of PTS between different bacterial groups	p-Value
The MIC values of PTS in 5 *C. freundii* CAR-S were not significantly different from that of the remaining 150 CAR-S bacteria	0.129[1]
The MIC values of PTS in 45 *A. baumannii* were significantly lower than those of the remaining 236 bacteria	<0.001
The MIC values of PTS in 40 *A. baumannii* CAR-R were significantly lower than those of the remaining 86 CAR-R bacteria	<0.001
The MIC values of PTS in 5 *A. baumannii* CAR-S were not significantly different from that of the remaining 150 CAR-S bacteria	0.704[1]
The MIC values of PTS in 34 *P. aeruginosa* were significantly higher than those of the remaining 247 bacteria	<0.001
The MIC values of PTS in 9 *P. aeruginosa* CAR-R were significantly higher than those of the remaining 117 CAR-R bacteria	<0.001[1]
The MIC values of PTS in 25 *P. aeruginosa* CAR-S were significantly higher than those of the remaining 130 CAR-S bacteria	<0.001
Comparison of the activity of PTSO between different bacterial groups	**p-Value**
The MIC values of PTSO in 102 *K. pneumoniae* were significantly lower than those of the remaining 179 bacteria	<0.001
The MIC values of PTSO in 42 *K. pneumoniae* CAR-R were significantly higher than those of the remaining 84 CAR-R bacteria	<0.001
The MIC values of PTSO in 60 *K. pneumoniae* CAR-S were significantly lower than those of the remaining 95 CAR-S bacteria	0.006
The MIC values of PTSO in 59 *E. coli* were significantly lower than those of the remaining 222 bacteria	<0.001
The MIC values of PTSO in 9 *E. coli* CAR-R were not significantly different from that of the remaining 117 CAR-R bacteria	0.281[1]
The MIC values of PTSO in 50 *E. coli* CAR-S were significantly lower than those of the remaining 105 CAR-S bacteria	<0.001
The MIC values of PTSO in 31 *E. cloacae* were not significantly different from those of the remaining 250 bacteria	0.944
The MIC values of PTSO in 21 *E. cloacae* CAR-R were not significantly different from that of the remaining 105 CAR-R bacteria	0.787
The MIC values of PTSO in 10 *E. cloacae* CAR-S were not significantly different from that of the remaining 145 CAR-S bacteria	0.928
The MIC values of PTSO in 10 *C. freundii* were significantly lower than those of the remaining 271 bacteria	0.031
The MIC values of PTSO in 5 *C. freundii* CAR-R were not significantly different from that of the remaining 121 CAR-R bacteria	0.624[1]
The MIC values of PTSO in 5 *C. freundii* CAR-S were significantly lower than those of the remaining 150 CAR-S bacteria	0.010[1]
The MIC values of PTSO in 45 *A. baumannii* were significantly lower than those of the remaining 236 bacteria	<0.001
The MIC values of PTSO in 40 *A. baumannii* CAR-R were significantly lower than those of the remaining 86 CAR-R bacteria	<0.001
The MIC values of PTSO in 5 *A. baumannii* CAR-S were not significantly different from that of the remaining 150 CAR-S bacteria	0.258[1]
The MIC values of PTSO in 34 *P. aeruginosa* were significantly higher than those of the remaining 247 bacteria	<0.001
The MIC values of PTSO in 9 *P. aeruginosa* CAR-R were significantly higher than those of the remaining 117 CAR-R bacteria	<0.001[1]
The MIC values of PTSO in 25 *P. aeruginosa* CAR-S were significantly higher than those of the remaining 130 CAR-S bacteria	<0.001

[1]The approximation to the form of the normal distribution becomes less robust at sample sizes smaller than 10.

PTSO (p = 0.150) against 126 CAR-R isolates compared to 155 CAR-S isolates. Similarly, there were no significant differences in the activity of these compounds when comparing CAR-R isolates to CAR-S isolates in each bacterial species. However, the MIC values of PTSO were significantly lower than those of PTS, regardless of whether the isolates were CAR-R or CAR-S. For each bacterial isolate tested, the MIC and MBC values for each organosulfur compound were either equal or differed by only one dilution (MBC ≥ MIC), indicating their bactericidal activity.

The MIC_{50} and MIC_{90} values of PTS in CAR-R bacteria ranged from 64 to 1024 mg/L and 128 to 2048 mg/L, respectively. *K. pneumoniae*, *E. coli*, and *A. baumannii* had significantly lower MIC values of PTS compared to the other bacteria. In contrast, *P. aeruginosa* exhibited the highest values, with an MIC_{50} of 1024 mg/L, MIC_{90} of 2048 mg/L, indicating significantly greater resistance to PTS than the

Table 4. Mean value of zone of bacterial growth inhibition (in mm) around disks of PTS, PTSO, and other antibacterial agents in carbapenem-resistant (CAR-R) and carbapenem-susceptible (CAR-S) bacteria

Bacterial species/antibacterial agent	Mean (±standard deviation)		Bacterial species/antibacterial agent	Mean (±standard deviation)	
	CAR-R	CAR-S		CAR-R	CAR-S
Klebsiella pneumoniae			*Escherichia coli*		
Amoxicillin/clavulanate	6.1 (±0.9)	20.2 (±3.0)	Amoxicillin/clavulanate	6.4 (±1.3)	16.7 (±4.5)
Piperacillin/tazobactam	9.0 (±2.6)	22.8 (±2.4)	Piperacillin/tazobactam	13.3 (±3.3)	23.4 (±3.4)
Cefotaxime	7.0 (±2.0)	26.8 (±0.9)	Cefotaxime	11.6 (±6.8)	27.8 (±1.6)
Ceftazidime	13.5 (±3.1)	26.0 (±1.5)	Ceftazidime	14.7 (±5.1)	26.5 (±2.9)
Cefepime	12.5 (±4.8)	29.3 (±1.0)	Cefepime	15.0 (±5.8)	29.4 (±1.8)
Imipenem	18.4 (±3.9)	24.0 (±0.8)	Imipenem	20.1 (±1.4)	27.5 (±1.9)
Ciprofloxacin	9.5 (±6.0)	26.7 (±1.3)	Ciprofloxacin	15.3 (±9.3)	29.3 (±2.7)
Amikacin	22.0 (±4.0)	21.1 (±1.1)	Amikacin	21.9 (±2.3)	22.1 (±1.1)
PTS (0.05 mg)	8.3 (±2.6)	10.8 (±2.1)	PTS (0.05 mg)	9.7 (±2.7)	12.1 (±2.4)
PTS (0.1 mg)	9.7 (±2.7)	11.5 (±2.0)	PTS (0.1 mg)	12.0 (±1.5)	14.0 (±2.7)
PTS (1 mg)	23.8 (±3.7)	25.1 (±4.2)	PTS (1 mg)	30.0 (±2.5)	30.6 (±3.5)
PTS (2 mg)	29.0 (±3.9)	28.4 (±3.2)	PTS (2 mg)	33.0 (±3.4)	34.3 (±3.3)
PTSO (0.05 mg)	11.7 (±2.4)	13.6 (±2.6)	PTSO (0.05 mg)	13.7 (±1.4)	15.3 (±2.0)
PTSO (0.1 mg)	14.3 (±2.6)	14.1 (±3.0)	PTSO (0.1 mg)	15.9 (±0.9)	16.0 (±1.9)
PTSO (1 mg)	28.0 (±3.9)	27.9 (±4.6)	PTSO (1 mg)	31.8 (±2.1)	32.0 (±3.9)
PTSO (2 mg)	30.6 (±3.5)	30.1 (±4.7)	PTSO (2 mg)	33.7 (±2.9)	35.1 (±3.3)
Enterobacter cloacae			*Citrobacter freundii*		
Amoxicillin/clavulanate	6.0 (±0.0)	10.0 (±2.1)	Amoxicillin/clavulanate	6.0 (±0.0)	16.0 (±0.7)
Piperacillin/tazobactam	12.2 (±2.5)	23.0 (±1.1)	Piperacillin/tazobactam	9.8 (±3.9)	25.8 (±0.8)
Cefotaxime	6.0 (±0.0)	26.5 (±0.5)	Cefotaxime	10.2 (±2.9)	28.4 (±1.1)
Ceftazidime	9.1 (±3.7)	25.0 (±0.0)	Ceftazidime	13.2 (±6.3)	26.0 (±0.7)
Cefepime	9.3 (±3.9)	28.0 (±0.0)	Cefepime	14.4 (±6.3)	29.6 (±1.1)
Imipenem	17.7 (±2.8)	23.5 (±0.5)	Imipenem	17.8 (±2.9)	24.0 (±0.7)
Ciprofloxacin	13.4 (±7.4)	29.5 (±1.6)	Ciprofloxacin	12.8 (±10.0)	31.6 (±1.1)
Amikacin	21.7 (±1.6)	20.5 (±0.5)	Amikacin	22.8 (±2.7)	22.6 (±1.1)
PTS (0.05 mg)	11.0 (±3.5)	11.5 (±1.6)	PTS (0.05 mg)	10.2 (±4.3)	11.8 (±1.1)
PTS (0.1 mg)	12.1 (±3.5)	12.5 (±1.6)	PTS (0.1 mg)	11.2 (±3.7)	12.2 (±1.1)
PTS (1 mg)	28.1 (±6.6)	26.5 (±0.5)	PTS (1 mg)	26.0 (±8.2)	28.2 (±0.5)
PTS (2 mg)	31.3 (±7.2)	32.0 (±0.0)	PTS (2 mg)	29.2 (±8.0)	30.6 (±0.9)
PTSO (0.05 mg)	13.4 (±4.0)	16.0 (±0.0)	PTSO (0.05 mg)	13.8 (±6.1)	14.0 (±0.7)
PTSO (0.1 mg)	14.9 (±4.1)	16.0 (±0.0)	PTSO (0.1 mg)	15.6 (±5.4)	15.0 (±0.7)
PTSO (1 mg)	29.1 (±7.3)	31.0 (±1.1)	PTSO (1 mg)	26.6 (±8.0)	29.0 (±1.0)
PTSO (2 mg)	33.5 (±6.6)	34.7 (±0.9)	PTSO (2 mg)	30.8 (±9.7)	31.8 (±0.8)
Acinetobacter baumannii			*Pseudomonas aeruginosa*		
Amoxicillin/clavulanate	6.0 (±0.0)	6.0 (±0.7)	Amoxicillin/clavulanate	6.0 (±0.0)	6.0 (±0.0)
Piperacillin/tazobactam	8.3 (±2.6)	20.2 (±1.1)	Piperacillin/tazobactam	13.6 (±4.3)	22.4 (±2.4)
Cefotaxime	6.0 (±0.0)	6.0 (±0.7)	Cefotaxime	6.0 (±0.0)	7.6 (±1.4)
Ceftazidime	7.1 (±1.3)	17.6 (±1.1)	Ceftazidime	10.7 (±4.3)	23.0 (±3.0)
Cefepime	7.7 (±2.4)	16.8 (±1.1)	Cefepime	8.0 (±3.8)	27.4 (±2.7)
Imipenem	12.5 (±2.2)	28.0 (±0.7)	Imipenem	9.6 (±3.7)	27.2 (±1.8)
Ciprofloxacin	6.0 (±0.0)	24.0 (±0.7)	Ciprofloxacin	20.7 (±11.2)	28.4 (±2.0)
Amikacin	9.0 (±5.7)	25.2 (±1.1)	Amikacin	20.0 (±5.1)	24.6 (±1.2)
PTS (0.05 mg)	15.9 (±4.8)	13.0 (±0.7)	PTS (0.05 mg)	6.0 (±0.0)	6.0 (±0.0)
PTS (0.1 mg)	16.8 (±4.1)	14.4 (±0.5)	PTS (0.1 mg)	6.1 (±0.3)	6.0 (±0.0)
PTS (1 mg)	26.7 (±3.2)	24.0 (±0.7)	PTS (1 mg)	11.9 (±2.8)	9.2 (±2.4)
PTS (2 mg)	29.5 (±3.2)	26.6 (±0.9)	PTS (2 mg)	13.3 (±2.5)	10.8 (±3.1)
PTSO (0.05 mg)	18.2 (±4.0)	15.6 (±0.9)	PTSO (0.05 mg)	6.1 (±0.3)	6.4 (±0.5)
PTSO (0.1 mg)	18.4 (±3.2)	16.0 (±0.7)	PTSO (0.1 mg)	7.1 (±0.9)	7.0 (±0.6)
PTSO (1 mg)	27.9 (±3.7)	25.0 (±0.7)	PTSO (1 mg)	14.2 (±2.9)	11.4 (±4.3)
PTSO (2 mg)	31.1 (±3.3)	28.6 (±0.9)	PTSO (2 mg)	15.6 (±2.9)	13.2 (±5.8)

other bacteria ($p < 0.001$). The MIC_{50} and MIC_{90} values of PTSO ranged from 32 to 256 mg/L and from 64 to 1024 mg/L, respectively. Notably, *P. aeruginosa* exhibited significantly higher resistance to PTSO (MIC_{50} = 256 mg/L, MIC_{90} = 1024 mg/L) ($p < 0.001$), while enterobacteria and *A. baumannii* had the lowest MIC values.

In CAR-S bacteria, the MIC_{50} and MIC_{90} values of PTS ranged from 64 to 1024 mg/L and 128 to 4096 mg/L, respectively. The MIC values were

significantly higher in *P. aeruginosa* compared to the other bacteria (MIC_{50} = 1024 mg/L, MIC_{90} = 4096 mg/L) ($p < 0.001$), while enterobacteria and *A. baumannii* showed significantly lower MIC values of PTS. The MIC_{50} and MIC_{90} values of PTSO ranged from 32 to 512 mg/L and from 64 to 1024 mg/L, respectively. It should be noted that *P. aeruginosa* was significantly more resistant to this compound than the other bacteria tested ($p < 0.001$). Additionally, PTS and PTSO were found to be less active against *P. aeruginosa* than any other Gram-negative bacteria ($p < 0.001$).

Table 4 presents a summary of the results of the antibacterial susceptibility tests conducted through disk diffusion. Inhibition zone growth was higher (>3 mm) among CAR-S isolates for all beta-lactam antibiotics and ciprofloxacin, regardless of the bacterial species. However, there were no significant differences found between CAR-R and CAR-S isolates for amoxicillin/clavulanate and cefotaxime in *A. baumannii* and *P. aeruginosa*. Both bacteria showed very small inhibition zones with differences of less than 3 mm. Additionally, no significant differences were observed between CAR-R and CAR-S in the diameter of the growth inhibition zone around the amikacin disk in enterobacteria. However, in *A. baumannii* and *P. aeruginosa*, the CAR-S isolates had the highest diameters, with a difference of over 3 mm compared to the CAR-R isolates. Similar to the results in Table 2, CAR-R isolates were found to be more resistant to ciprofloxacin than CAR-S isolates when tested using the disk diffusion method. Furthermore, there were no significant differences in the susceptibility of *Enterobacteriaceae* to amikacin whether they were CAR-R or CAR-S. However, non-fermented Gram-negative bacteria, particularly *A. baumannii*, exhibited higher resistance to amikacin among CAR-R isolates.

There were no significant differences in the bacterial growth inhibition zone (differences less than 3 mm) between CAR-R isolates and CAR-S isolates at any of the concentrations of PTS and PTSO tested. This suggests that these compounds have similar activity against all bacterial species, regardless of the presence or absence of carbapenemases. Moreover, it was observed that PTSO exhibited greater activity than PTS. This was supported by the fact that, when using the same concentration of substance, the diameters were consistently larger with PTSO. However, it is important to note that in some cases the differences were less than 3 mm (see Fig. 1). Similarly to microdilution, the disk

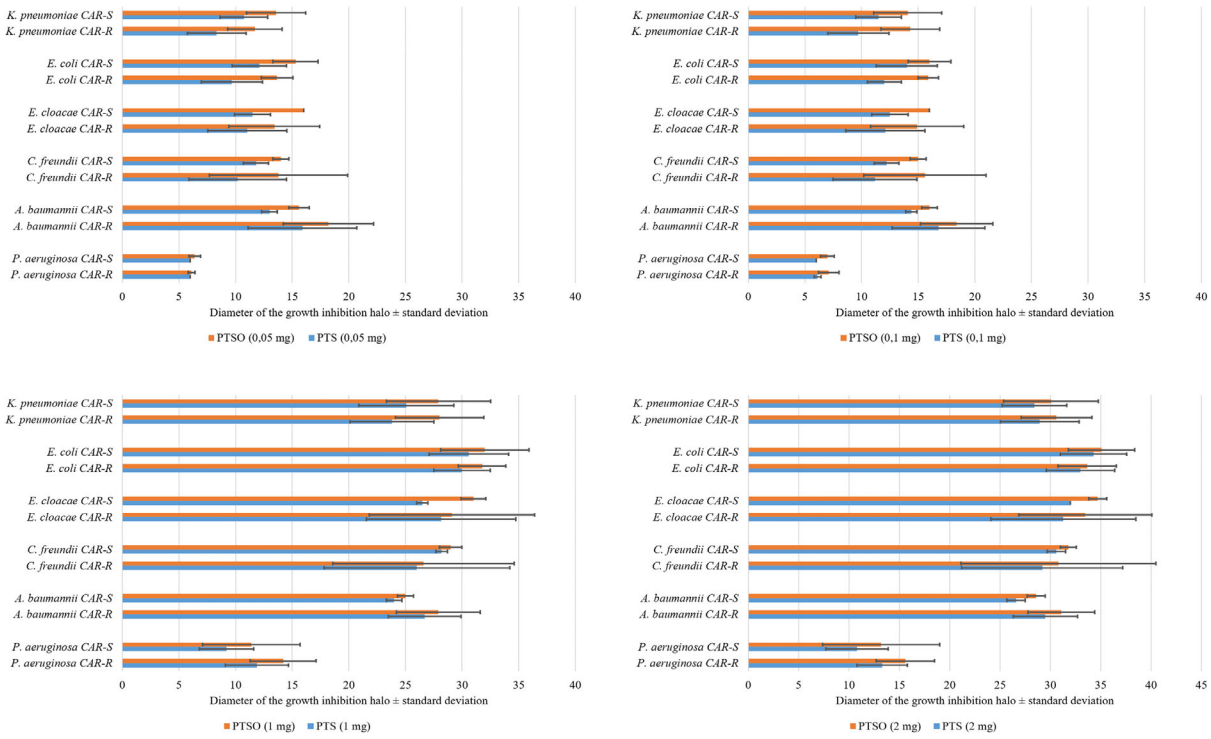

Fig. 1. Comparison of the mean value (in mm) of the zone of bacterial growth inhibition around the disks of PTS and PTSO for each bacterial species.

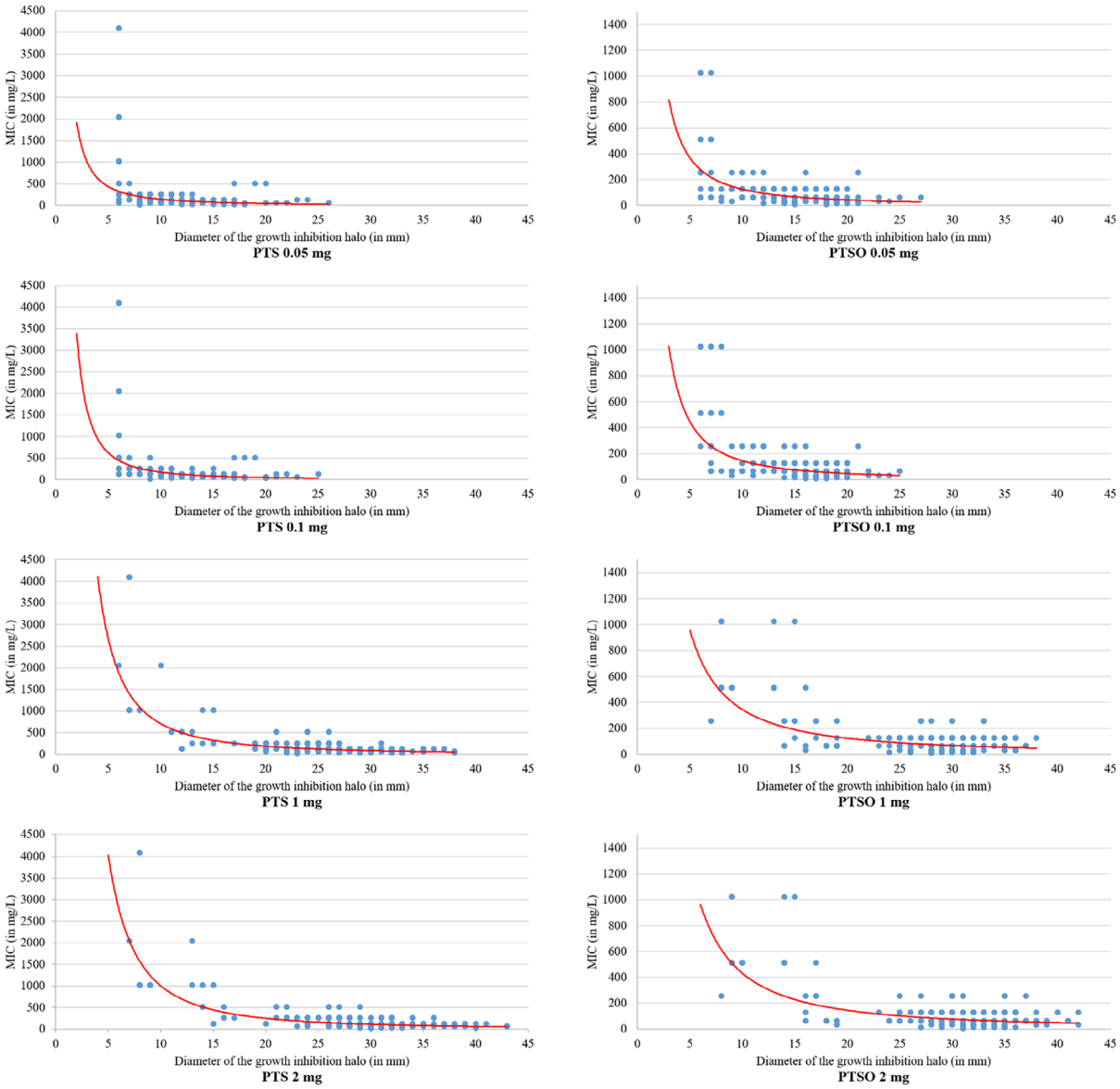

Fig. 2. Relationship between the diameter of the bacterial growth inhibition zone around the PTS and PTSO disks (horizontal axis) and the MIC values (vertical axis) obtained in the total of clinical bacterial isolates.

diffusion method demonstrated lower activity of PTS and PTSO against *P. aeruginosa* compared to other bacteria.

Figure 2 shows the correlation between the bacterial growth inhibition zones for each concentration of PTS and PTSO disks (horizontal axis) and MIC values (vertical axis) obtained for all bacteria. The larger the diameter of the inhibition zone, the lower the MIC value obtained. The model fits best for the higher concentrations tested (1–2 mg), making them the most suitable for studying the susceptibility of bacteria to these compounds. For PTS disks, bacterial growth inhibition zone diameters greater than 15 mm correspond to MIC values of 512 mg/L or lower when using concentrations of 1 or 2 mg. For PTSO disks, inhibition zone diameters greater than 15 mm (using 1 or 2 mg concentration) correspond to a MIC value of 256 mg/L or lower, demonstrating the superior activity of PTSO compared to PTS. Diameters greater than 35 mm are indicative of MIC values of 128 mg/L or lower for both substances.

DISCUSSION

Beta-lactams are the largest family of antibiotics and are widely prescribed in clinical practice due to their broad spectrum of activity. However, bacteria resistant to this group of antibiotics are emerging worldwide, particularly in the case of carbapenem antibiotics, which are typically used as a last resort to treat infections caused by Gram-negative bacteria. This severely limits the therapeutic options available for such infections. Several mechanisms can lead to high levels of resistance to carbapenems, including enzyme production, efflux pumps, and porin mutations. However, the most common mechanism is the presence of carbapenemases [16].

In this study, most of the carbapenemases currently described in Gram-negative bacteria were detected in the 126 clinical CAR-R isolates. *Klebsiella pneumoniae* carbapenemases (KPC) are serine carbapenemases belonging to Ambler molecular class A. Although they were originally discovered in *K. pneumoniae*, they can also be found in other Gram-negative bacteria. The *blaKPC* gene is located in a transposon with the ability to insert into plasmids, allowing cotransfer of antibiotic resistance mechanisms such as fluoroquinolones or aminoglycosides, among others. Active-on-imipenem carbapenemase (IMP), Verona integron-encoded metallo-betalactamase (VIM), and New Delhi metallo-betalactamase (NDM) are metallo-beta-lactamases belonging to the Ambler molecular class B. IMP and VIM were first discovered in *P. aeruginosa*, while NDM was first described in *K. pneumoniae*. Mobile genetic elements have facilitated the rapid spread of OXA-type carbapenemases to other Gram-negative bacteria, particularly among members of the Enterobacterales. These enzymes are often associated with multidrug-resistant bacteria. OXA-type carbapenemases belong to class D, of which OXA-23 and OXA-48 are the most common. In European countries, especially in Spain, OXA-48 is significantly associated with nosocomial outbreaks, more than any other enzyme. Although *Acinetobacter* spp. are the main bacteria producing OXA-carbapenemases, their presence in enterobacteria has increased significantly. In particular, OXA-48 *K. pneumoniae* is frequently isolated [6, 17].

No carbapenemase-producing bacteria were reported in Spain until 2005. In the following years, only sporadic cases of VIM- and IMP-producing bacteria were reported [18]. However, the situation has recently worsened with a significant increase in the number of cases worldwide. OXA-48, followed by VIM-1, has become more widespread and, in particular, the number of hospitals affected by serious outbreaks has increased throughout the Spanish territory [19, 20]. In Spain, OXA-48 is currently the most common enzymatic mechanism of carbapenem resistance [21]. This is particularly true in Granada [22], as demonstrated by the clinical isolates selected for this study.

A promising strategy to reduce the impact of infections caused by multidrug-resistant bacteria is the search for new molecules with antibiotic potential [23]. Organosulfur compounds derived from *Allium* plants, such as PTS and PTSO, are effective alternatives [24]. These compounds have current applications in poultry, aquaculture, and the food industry [25, 26]. They may also have potential for use in human clinical practice due to their traditional use for centuries as antimicrobial agents [10]. Although the mechanism of action of these substances is not fully understood, they are highly permeable across phospholipid membranes and interact with and inhibit some thiol-dependent enzymatic systems, RNA polymerase, and RNA synthesis [27, 28].

Nevertheless, one of the main problems with testing antimicrobial activity in natural products is the lack of standardization, which makes it difficult to quantify and compare the effects of these substances. This is particularly important when comparing them to commonly used antimicrobials in human therapy. Therefore, the aim of the current study was to evaluate the activity of PTS and PTSO against a range of susceptible and resistant bacteria isolated from human clinical samples. We also compared the *in vitro* activity of different antibiotics against these microorganisms. The assays were performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [29]. Although there are no breakpoints to determine bacterial susceptibility or resistance to PTS or PTSO, performing the assay under the same conditions as other antibiotics and quantifying the antibacterial activity in terms of MIC and/or diameter of the zone of inhibition of bacterial growth allows comparisons between them.

The results of the present study show that both PTS and PTSO have bactericidal activity against Gram-negative bacteria of the *Enterobacteriaceae* group and *A. baumannii*, irrespective of the presence of enzymatic resistance mechanisms to carbapenems. Previous studies have shown that these compounds have significant antibacterial activity against multidrug-resistant bacteria isolated from human samples [14, 15].

In contrast to these findings, the compounds tested showed lower activity against *P. aeruginosa*, regardless of the bacterium's resistance to carbapenems, as previously demonstrated [14, 15]. *P. aeruginosa* has an outer membrane with low permeability and several active efflux systems that confer innate resistance to multiple antibiotics [9].

When PTS and PTSO come into contact with these bacteria, they may be affected by these mechanisms. Further studies are required to determine the appropriate mechanisms involved in this increased resistance [14].

Both this and previous studies [14, 15], have shown that PTSO is significantly more active than PTS, probably due to its greater stability. Allicin (diallyl thiosulfinate), the primary organosulfur compound found in *Allium*, is easily degraded, which rapidly reduces its antibacterial activity. Similarly, another thiosulfinate (PTS), although more stable than allicin, undergoes dismutation or disproportionation reactions resulting in the most stable molecule with greater antibacterial activity [27].

The study results indicate that while the MIC values of these compounds are higher than those of commonly used antibiotics in susceptible bacteria, the activity of PTS and especially PTSO against Gram-negative bacteria resistant to conventional antibiotics is promising. Natural compounds that are safe and easy to administer offer the potential to treat infections. However, proper formulation is critical to ensure efficacy. It is important to maintain a concentration of the substance at the site of infection that exceeds the MIC values against the bacteria causing the infection. Therefore, before safe clinical use can be considered, further studies mainly focus on the evaluation of pharmacokinetics and toxicological properties [30, 31]. It will be necessary to determine the appropriate routes of administration of the compounds and their efficacy in human trials. Finally, the concentrations achieved in various tissues and fluids should be further evaluated [14].

MATERIALS AND METHODS

Bacterial isolates

A total of 126 clinical bacterial isolates resistant to carbapenems (CAR-R) were selected for this study. These included 42 *K. pneumoniae*, 9 *E. coli*, 21 *E. cloacae*, 5 *C. freundii*, 40 *A. baumannii*, and 9 *P. aeruginosa*. In addition, 155 isolates susceptible to carbapenems (CAR-S) were selected. These included 60 *K. pneumoniae*, 50 *E. coli*, 10 *E. cloacae*, 5 *C. freundii*, 5 *A. baumannii*, and 25 *P. aeruginosa*. The clinical isolates analyzed in this study were selected from urine cultures obtained for urinary tract infection analysis in the hospital microbiology laboratory and had a significant bacterial count (isolation $\geq 10^5$ colony-forming units/mL).

All isolates were identified using the automated MicroScan system (Beckman Coulter, Brea, California, USA) or by mass spectrometry (MALDI-TOF®, Bruker Daltonik GmbH, Bremen, Germany) as part of the routine microbiology laboratory workup [32]. Carbapenemase production was detected using Rapidec® Carba NP (bioMerieux, Madrid, Spain), NG-Test® Carba-5 (NG Biotech, Guipry-Messac, France), and OXA-23 K-Set (CorisBioConcept, Gembloux, Belgium). The loss of the OprD porin in a single isolate was confirmed by the reference laboratory of the Regional Integrated Program for the Prevention and Control of Healthcare-Related Infections and Appropriate Use of Antibiotics (Spanish acronym: PIRASOA) in Sevilla (Spain).

Minimum inhibitory concentration and minimum bactericidal concentration determination

To determine antibacterial susceptibilities, all 281 isolates underwent a broth microdilution assay in Cation-Adjusted Mueller–Hinton Broth (CAMHB) according to CLSI guidelines [29].

All antibiotics were purchased from Sigma-Aldrich (Madrid, Spain) and dissolved according to the manufacturer's recommendations. Both PTS and PTSO (97% purity) were provided by Enzim-Orbita Agroalimentares LDA (Tavira, Portugal) and dissolved in Tween-80 to a final concentration of 500 g/L. The biosynthesis of PTS and PTSO starts with propiin, an amino acid derived from L-cysteine found in *Allium* species. The first step in the biosynthesis is the formation of a sulfenic acid, which is highly reactive and immediately produces PTS by a condensation reaction. In the final step, the oxidation of PTS induces its dismutation to PTSO and propyl disulfide, which can be oxidized and converted to PTSO, thus completing the oxidation of PTS to PTSO.

Broth microdilution tests were performed using 96-well round-bottom microtiter plates. The bacterial cell suspension was adjusted to a final concentration of 1×10^5 CFU/mL in each well. Each plate contained negative controls consisting of medium only and 11 serial twofold dilutions of each antibiotic, either PTS or PTSO. Positive controls, consisting of bacterial suspension without antibiotics, were included in a separate round-bottom plate.

The antibiotics were tested in the following concentration ranges (in mg/L): amoxicillin/clavulanate (2/1–2048/1024), piperacillin/tazobactam (4/4–4096/4), cefotaxime (0.5–512), ceftazidime (2–2048), cefepime (1–1024), imipenem (0.25–256), ciprofloxacin (0.125–128), and amikacin (2–2048). The concentration range of PTS or PTSO was 8–8192 mg/L. Therefore, the final concentration of Tween-80 in the wells was less than 1%.

The minimum inhibitory concentration (MIC) is the lowest amount of antibiotic that completely inhibits visible growth of a microorganism after overnight incubation. Isolates were classified as susceptible, intermediate or resistant based on the CLSI interpretation categories and MIC breakpoints [29]. Bacteria were grouped into two categories based on their MIC data: susceptible or resistant (intermediate and resistant organisms were grouped together). There are no clinical breakpoints for the susceptibility patterns of PTS or PTSO. MIC_{50} and MIC_{90} values were defined as the lowest concentration of antibiotic at which 50% and 90% of isolates were inhibited, respectively.

For minimum bactericidal concentration (MBC) testing, 0.1 mL of broth from 1 to 4 wells with no growth was plated on Columbia Agar without antibiotics and incubated overnight at 36 ± 1°C. The MBC was determined as the highest dilution that did not produce a single bacterial colony on the agar plates. The MBC_{50} and MBC_{90} values represent the concentration of antibiotic required

to eliminate 50% and 90% of the isolates, respectively. Depending on the MBC/MIC ratio, allium extracts with values greater than 2 or between 1 and 2 were considered bacteriostatic or bactericidal, respectively.

Kirby–Bauer disk diffusion method

The Kirby–Bauer disk diffusion method was performed on Mueller–Hinton agar plates using a McFarland 0.5 bacterial inoculum. The following antibiotic disks and concentrations were used: amoxicillin/clavulanate (20/10 μg), piperacillin/tazobactam (100/10 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg), imipenem (10 μg), ciprofloxacin (5 μg), and amikacin (30 μg). PTS (0.05 mg, 0.1 mg, 1 mg, and 2 mg) and PTSO (0.05 mg, 0.1 mg, 1 mg, and 2 mg) were also used.

It was determined that a difference of 3 mm or more in the mean values of the zone of bacterial growth inhibition around the disks of antibiotics, PTS and PTSO, when comparing CAR-R and CAR-S isolates, indicates significant differences in the activity of the tested substance.

In accordance with CLSI guidelines, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 strains were used for as quality control in all procedures.

Statistical analysis

The Mann–Whitney *U*-test was used to compare the distribution of MIC values of PTS and PTSO between different bacterial species and between CAR-R and CAR-S isolates. p-Values <0.05 were considered statistically significant for all comparisons.

CONCLUSIONS

In vitro bactericidal activity of PTS and PTSO was observed against clinical isolates of *Enterobacteriaceae* and *A. baumannii* that were resistant to carbapenem antibiotics. The activity against susceptible isolates was not significantly different. However, the activity of both compounds against *P. aeruginosa* was significantly lower compared to the other bacteria, regardless of their carbapenem resistance. In all cases, the antibacterial activity of PTSO was significantly higher than that of PTS. These results provide valuable information on the potential use of these compounds in preventing or treating human infections, especially in cases where current therapeutic options are limited. However, it is important to ensure safe clinical use by including them appropriate formulations according to their route of administration and that safe clinical use is ensured.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLOSURE STATEMENT

Affirmative.

DATA AVAILABILITY STATEMENT

The data presented in this study are available in the main text.

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