ORIGINAL ARTICLE

Evaluation of a new combination: ceftriaxone-disodium edetate-sulbactam as a broad-spectrum option for multidrug-resistant bacterial infections

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ABSTRACT

Background: The presence of numerous antibiotic-resistance mechanisms in Gram-positive and Gram-negative bacteria is a global concern, which is further complicated by emergence of newer mechanisms in recent years. Few new compounds are in the production-pipeline that show potential for usage as antimicrobial agents. Antibiotic adjuvant ceftriaxone-disodium edetate-sulbactam, available under tradename Elores™, showed potential in this study by demonstrating antimicrobial activity against various multidrug-resistant bacteria.

Methods: In this prospective in-vitro study, we tested the antimicrobial activity of Elores™ against a battery of clinical Gram-positive and Gram-negative bacterial isolates, including antibiotic-susceptible and antibiotic-resistant bacteria such as ESBL-producing Enterobacterales (ESBLPE), carbapenem-resistant Enterobacterales (CRE), Enterobacterales showing colistin-resistance (ECR), methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant Enterococci (VRE).

Results: Elores™ showed excellent activity against the tested Gram-positive and Gram-negative bacterial species, including some highly resistant species such as ESBL-producers, CRE, species resistant to colistin, MRSA and VRE. Elores™ was non-inferior to tigecycline in VRE isolates and non-inferior to colistin in *Escherichia coli*, *Citrobacter* species, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. However, in *Klebsiella* species the activity of Elores™ was notably better than colistin.

Conclusions: In addition to activity against ESBL-producers and CRE, the activity of Elores™ against colistin-resistant Enterobacterales, MRSA and VRE showed promise, indicating its use as a potential candidate for empirical therapy due to its high activity against multidrug-resistant Gram-positive and Gram-negative bacteria.

Keywords: Elores™, antibiotic-resistance mechanisms, Enterobacterales.

N Z J Med Lab Sci 2020; 74: 22-26

INTRODUCTION

Antimicrobial resistance has dramatically increased in recent years to an alarming extent, subsequent to which global initiatives were called upon from different forums to curb this threatening issue, including the endorsement of a global action plan by the World Health Organization (WHO) to curb antimicrobial resistance (1). Recently, in a series (Antimicrobials: access and sustainable effectiveness) published by the *Lancet* had emphasised the need for policy interventions to combat emerging global burden of resistance (2). Confronted with this burning issue, other leading journals also emphasised a pressuring need to develop newer antibiotics (3). The rates of antimicrobial resistance vary geographically, which in many cases is a reflection of selection pressure due to antibiotic prescribing habits. However, in all of these resistance cases the mechanisms may be multiple, including reduced permeability to antibiotics, increased efflux pumps, changes in antibiotic targets by mutation, or modification of target enzymes; all of which are prevalent globally.

However, the spread of the resistance genes through various mobile genetic elements is the common mechanism of acquired resistance (4,5). Few new antibiotics are currently in the pipeline which would otherwise raise some hope in curbing life threatening infections caused by extensively resistant organisms. In such a situation, if a novel compound shows promising results against resistant bacteria, it brings hope for future treatment options. Elores™ (ceftriaxone- disodium edetate- sulbactam) is a promising new agent which is currently patented in many countries, including in the U.S. (the details of the patent numbers in respective countries are given in the respective methodology section). Recent published data projects this new antibiotics adjuvant as a carbapenem-sparing drug, mainly against Gram-negative bacteria, and demonstrated benefits of using this novel compound empirically in severe illnesses, including ventilator associated pneumonia (6).

However, the potential of Elores™ has not been thoroughly explored against Gram-positive extensively drug-resistant bacteria such as MRSA and VRE, nor against Gram-negative bacteria resistant to colistin, which is generally used as a last resort antibiotic. In this study we evaluated the antimicrobial potential of this antibiotic adjuvant against multidrug-resistant clinical Gram-positive and Gram-negative bacterial isolates, including ESBL-producing- and carbapenem-resistant-Enterobacterales, colistin-resistant Enterobacterales, methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococci.

METHODS

Study type

This was a prospective in-vitro study performed on routine clinical samples received for culture and sensitivity at the Microbiology Laboratory of the Jawaharlal Nehru Medical College & Hospital Aligarh, India. Ethical clearance was obtained from the Institutional Ethical Committee of the Jawaharlal Nehru Medical College & Hospital.

Elores™ patent details

The patent information provided by the source company Venus Remedies, India is as follows: 236996 (India), 2007/4394 (South Africa), 91204 (Ukraine), 2397768 (Russia), 279582

(Mexico), 2005310888 (Australia), 555075 (New Zealand), 8273732; 13/626, 236 (USA), 10-1244362 (South Korea), 5269415 (Japan), EP1841432 (Europe).

Patients and clinical samples

A total of 111 patients were included in this study from which the respective number of bacterial isolates, including various Gram-negative bacterial species, MRSA and VRE, were obtained after routine culture and sensitivity. These bacterial isolates were further tested for antibacterial activity against Elores™ discs. Gram-negative bacterial isolates were obtained from 67 clinical samples: pus-44; tracheal aspirate-6; sputum-5, blood-4; broncho-alveolar lavage and semen-2 each; cerebrospinal fluid, cervical swab, urine and vaginal swab-1 each. Twenty-seven VRE and 17 MRSA were obtained from urine, pus, blood and abdominal drain specimens. All 27 VRE and 17 MRSA were checked by molecular studies (PCR) for *van*A, *van*B and *mec*A genes respectively as per published procedures (7,8). Sixty three out of 67 Gram-negative isolates were ESBL producers, as determined by the Clinical Laboratory Standards Institute combination disc method (12). Representative isolates of resistant Gram-negative bacterial species were tested for respective molecular mechanism of resistance ($bla_{CTX\text{-}M}$, bla_{ampC} and $bla_{NDM\text{-}1}$) as per the procedures published elsewhere (5,9-11).

Bacterial isolates

The following Gram-negative isolates were obtained from 67 clinical samples: *E. coli* (n=38), *Citrobacter* spp (n=10), *Klebsiella pneumoniae* (n=6), *Pseudomonas aeruginosa* (n=6), *Acinetobacter baumannii* (n=4) and *Klebsiella oxytoca* (n=3). From the remaining 44 samples MRSA and VRE were obtained (MRSA= 17 and VRE= 27 isolates). Ten representative samples that were phenotypically ESBL were genotypically confirmed by the presence of CTX-M gene. Six representative samples, three *Pseudomonas* species and three *Enterobacterales* were tested for NDM-1, but none showed the presence of NDM-1 gene.

Routine culture identification/ sensitivity and Elores™ discs

The bacterial isolates were tested for identification and sensitivity by Kirby-Bauer method and the antibiotic susceptibility results were interpreted as per standard procedures (12,13). For quality control, *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (*NCTC 6749)* were used. Representative isolates of Gram-negative bacterial species and all the MRSA and VRE strains were confirmed by automated Vitek-2 compact system (Biomerieux-Diagnostics, USA). For antibiotic susceptibility testing of Gram-negative bacterial species (except *Pseudomonas* spp) the following antibiotics and concentrations were used: amikacin (30 µg), amoxicillin-clavulanate (20/10 µg), cefixime (5 µg), ceftriaxone (30 µg), ceftriaxone-sulbactam (30/15 µg), cotrimoxazole (1.25/23.75 µg), piperacillin-tazobactam (100/10 µg), levofloxacin (5 µg), meropenem (10 µg), colistin (10 µg) and Elores™ (30/15 µg). The antibiotics used for *Pseudomonas* species were: piperacillin-tazobactam (100/10 µg), amikacin (30 µg), aztreonam (30 µg), cefepime (30 µg), ceftazidime (30 µg), meropenem (10 µg), gentamicin (10 µg), levofloxacin (5 µg), colistin (10 µg) and Elores™ (30/15 µg). The antibiotic panel used for MRSA strains was: azithromycin (15 µg), amoxicillin-clavulanate (20/10 µg) (interpreted according to EUCAST) [14], levofloxacin (5 µg), cotrimoxazole (1.25/23.75 µg), clindamycin (2 µg), amikacin (30 µg), cefoxitin (30 µg), vancomycin (30 µg) and Elores™ (30/15 µg). And, for VRE, the following antibiotics were tested: benzyl penicillin (10 units), high content gentamicin (120 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), erythromycin (15 µg), linezolid (30 µg), teicoplanin (30 µg), vancomycin (30 µg), tetracycline (30 µg), nitrofurantoin (300 µg), tigecycline (15 µg) and Elores™ (30/15 µg). Resistance to colistin in Gram-negative bacteria and vancomycin in MRSA and VRE was further confirmed by the automated Vitek-2 system.

In-vitro susceptibilty testing of Elores™ discs

Antibiotics susceptibility of Elores™ discs against the Gram-negative and Gram-positive (MRSA and VRE) isolates was performed by the Kirby-Bauer method (13). Antibiotics discs (45; 30/15 µg) of antibiotic-adjuvant ceftriaxone-disodium edetate-sulbactam were procured from Abtek Biologicals Ltd, Liverpool, United Kingdom. Results were interpreted as per the manufacturer's instructions.

RESULTS

The characteristics of the resistance mechanisms of the Gram-negative isolates tested are given in Tables 1 and 2.

Table 1. Distribution of ESBL-producing Gram-negative isolates.

Organisms		Phenotypic detection for ESBLs	
		Test performed on no. of isolates	ESBL detected in no. of isolates
Enterobacterales		57	55
	Escherichia coli	38	37
	Citrobacter species	10	10
	Klebsiella species	9	8
Pseudomonas species		6	5
Acinetobacter species		4	3

Table 2. Genotypic characterisation of representative isolates.

Isolate no.	Organism	Gene detected
40	*Klebsiella* species	$bla_{CTX\text{-}M}$, bla_{TEM}, bla_{SHV}
11	*Escherichia coli*	$bla_{CTX\text{-}M}$, bla_{ampC}
3	*Klebsiella* species	$bla_{CTX\text{-}M}$, bla_{TEM}, bla_{SHV}
61	*Escherichia coli*	$bla_{CTX\text{-}M}$, bla_{ampC}
36	*Escherichia coli*	$bla_{CTX\text{-}M}$, bla_{ampC}
57	*Escherichia coli*	$bla_{CTX\text{-}M}$, bla_{ampC}
56	*Klebsiella* species	$bla_{CTX\text{-}M}$, bla_{ampC}
59	*Escherichia coli*	$bla_{CTX\text{-}M}$, bla_{ampC}
55	*Escherichia coli*	--------
47	*Citrobacter* species	$bla_{CTX\text{-}M}$
54	*Pseudomonas* species	bla_{SHV}
58	*Pseudomonas species*	----------
38	*Pseudomonas* species	-----------

Antibiotics susceptibility profile and Elores™ activity

Gram-negative bacterial isolates tested in this study were multidrug-resistant including some isolates, such as *Acinetobacter baumannii*, which were resistant to all of the tested antibiotics except colistin (Table 1). Elores™ was non-inferior to colistin in Gram-negative bacterial species (*E. coli*, *Citrobacter* species, *A. baumannii* and *P. aeruginosa*). However, in *Klebsiella* species, the activity of Elores™ was better than colistin (Table 3). All of the vancomycin-resistant *Enterococci* were also resistant to fluoroquinolones tested.

Alarmingly 22.2% (6/27) of VRE were resistant to linezolid. All of the VRE isolates were susceptible to tigecycline, and Elores™ was found non-inferior to tigecycline against VRE. Interestingly, Elores™ was active against the bacterial isolates which harbored complex molecular resistance mechanisms. The detailed antibiotic susceptibility pattern of various antibiotics, including Elores™, against Gram-negative and Gram-positive isolates (MRSA and VRE) is shown in Table 1. The representative in-vitro susceptibility results of elores discs against various bacterial species and the representative resistance mechanisms: ESBL ($bla_{CTX\text{-}M}$/ bla_{ampC}), CRE ($bla_{NDM\text{-}1}$), VRE (bla_{vanA}) and MRSA (bla_{mecA}) are shown in Figure 1. Gram-positive isolates (MRSA and VRE) are shown in Table 3.

Table 3. Antibiotic susceptibility profile (including Elores™) against bacterial species tested.

Organism (n) and antibiotics tested	Resistant percentage (n)	Sensitive percentage (n)
***Enterobacterales* (57)**		
Amikacin	47.37% (27)	52.63 % (30)
Meropenem	49.12% (28)	50.88% (29)
Colistin	7.02% (4)	92.98% (53)
Elores	0% (0)	100% (57)
Acinetobacter (4)		
Amikacin	100 % (0)	0 % (0)
Meropenem	100 % (0)	0 % (0)
Colistin	25 % (1)	75% (3)
Elores	0 % (0)	100% (0)
***Pseudomonas* (6)**		
Amikacin	50 % (3)	50% (3)
Meropenem	83.33 %(5)	16.67% (1)
Colistin	0% (0)	100% (0)
Elores	0% (0)	100 % (0)
VRE (27)		
Ceftriaxone	100 % (27)	0% (0)
Ceftriaxone+sulbactum	100 % (27	0% (0)
Elores	3.70% (1)	96.30% (26)
Linezolid	22.22% (6)	77.78% (21)
Levofloxacin	100 % (0)	0 % (0)
Erythromycin	96.30% (26)	3.70% (1)
Tetracycline	51.85% (14)	48.15 % (13)
Tigecycline	0 % (0)	100 % (27)
MRSA (17)		
Azithromycin	82.35% (14)	17.65% (3)
Levofloxacin	70.59% (12)	29.41 % (5)
Cotrimoxazole	58.82% (10)	41.18% (7)
Clindamycin	35.29% (6)	64.71% (11)
Amikacin	17.65% (3)	82.35 % (14)
Vancomycin	0% (0)	100 % (17)
Elores	0% (0)	100 % (17)

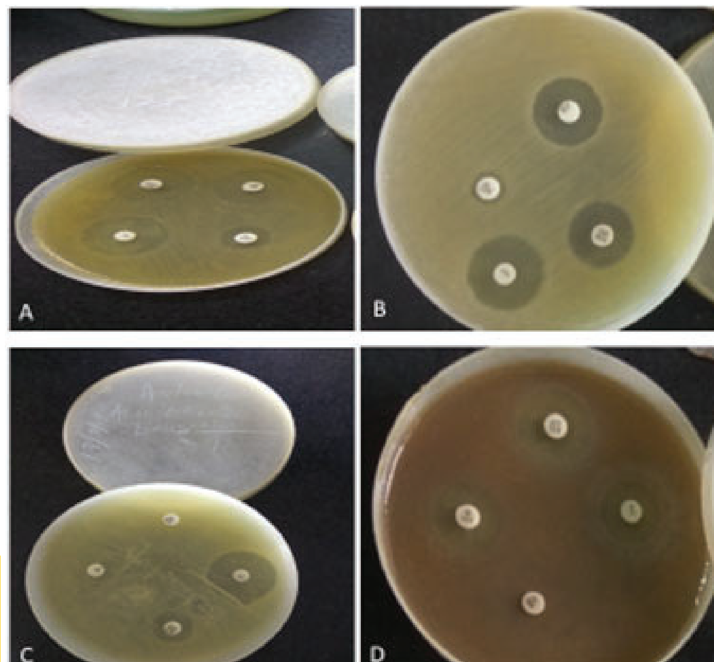

Figure 1. Antibacterial activity of Elores against representative *E. coli* (A), *Klebsiella pneumoniae* (B), *Acinetobacter baumannii* (C) and *Pseudomonas aeruginosa* (D).

DISCUSSION

Antibiotic resistance in Gram-negative bacteria has increased dramatically over the past few decades and the most common mechanism is by the production of beta-lactamases such as extended-spectrum beta-lactamases (15,16). Due to this increase in ESBL-producing isolates in clinical settings, carbapenems were considered empirically or as a tailored-down therapy. However, in recent years a significant volume of clinical isolates have demonstrated the presence of metallo-beta-lactamases, thus causing treatment failure to prescribed carbapenems (17). Due to this increase of emerging extended-resistant isolates there has been a pressured need to prescribe reserved antibiotics such as tigecycline and colistin. However, reports of resistance to these reserved drugs have also emerged in recent years (9,18-23). Emergence of resistance even to these reserved drugs has landed us in a very critical situation seeing that there are not many investigative drugs available in the antibiotic armamentarium (17). Said that, researchers recently have tried exploring potential of using potentiators of the already existing antibiotics in the form of antibiotic-adjuvants (17). Antibiotic-adjuvants refer to molecules which usually do not have antibiotic activity. These adjuvants are combined with antibiotics in order to potentiate the overall activity of the antibiotic-adjuvant entity through various mechanisms as discussed below. One recently developed example is ceftriaxone- disodium edetate- sulbactam (Elores™) which is prepared by combining:

- Antibiotic = ceftriaxone
- ESBL-inhibitor = sulbactam
- Adjuvant = disodium edetate

The antibiotic adjuvant Elores™ was initially patented by the Companies and Intellectual Property Registration Office (CIPRO), South Korea and was marketed back in 2013 by a Korean Pharma company, Goodwills Co (24). Clinical trials have suggested the clinical and microbiological efficacy of Elores™ in ESBL-producing Gram-negative pathogens and few Gram-positive pathogens showing clinical cure rate of as high as 80.3% as opposed to patients treated with ceftriaxone, which showed a cure rate of 30.8% (24). The microbiological efficacy in terms of bacterial eradication was reported as high as 85.3% in contrast to ceftriaxone alone (23.1%) (25).

Various possible mechanisms have been proposed for enhanced activity of Elores™ (6), such as:

- Ceftriaxone, sulbactam and EDTA acting synergistically.
- Enhanced activity of the antibiotic-adjuvant due to chelation of divalent ions by EDTA.
- Increased penetration of the antibiotic-adjuvant due to alteration of outer membrane permeability of bacteria.

However, we speculate that though the exact mechanism is not yet clear, it could be a complex mechanism such as the ones stated above plus the individual activities of sulbactam and EDTA, such as:

- Sulbactam showing intrinsic antibacterial activity against certain bacterial species, for instance Acinetobacter (26).
- Antimicrobial activity of native EDTA against pathogenic bacteria (27,28).

It is noteworthy that an anti-biofilm activity of EDTA has recently been reported (27,28). These studies would suggest that incorporating potentiators, such as EDTA, to the existing antibiotic armamentarium could be utilised as an alternative approach to combat antibiotic resistance; at least until we receive newer compounds showing efficient antibacterial activity against extensively- or pan-resistant organisms. In this era of emerging extensive- or pan-resistant organisms, combined with the paucity of available alternatives, the idea of using carbapenem-sparing agents is well understood. Thus, Elores™ could widely be considered as an approach to empirical therapy as evident from its activity, in this study, against a wide range of extensively resistant Gram-positive and Gram-negative bacteria. Also, as projected in earlier studies, Elores™ is not only a carbapenem-sparing option, rather it could be utilised as the sparer of last resort antibiotics, such as colistin and tigecycline, as it was found to be non-inferior to tigecycline in VRE isolates and non-inferior to colistin in Gram-negative bacterial isolates. The results of this study, especially the activity of Elores™ against colistin-resistant *Enterobacterales*, MRSA and VRE, warrants the need for large scale clinical trials on patients infected with these resistant organisms in order to validate its clinical efficacy against infections caused by these life-threatening bacteria.

One major limitation of our study was that we did colistin susceptibility by disc diffusion and VITEK automated method but no microbroth dilution or detection of *mcr-1* gene was done. Another limitation of our study is that the interpretation for Elores™ was by manufacturer and not CLSI and some groups of organisms tested are quite small, e.g. only four *A. baumannii* and nine *P. aeruginosa*. We also did not test for other common carbapenemases types prevalent in India, apart from NDM-1.

ACKNOWLEDGEMENTS

Authors wish to thank Venus Remedies for providing the antibiotic adjuvant (Elores™) discs.

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