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Research Article

ARBEKACIN – A RAY OF HOPE TO FIGHT AGAINST MDR AND XDR GRAM-NEGATIVE BACTERIA IN A SCIENTIFIC AND COST-EFFECTIVE WAY IN INDIAN SCENARIO

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ABSTRACT

Objective: The objective of the study was to see the *in vitro* activity of arbekacin, a novel aminoglycoside, against multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacilli (GNB) so that it can become a good alternative as empirical treatment for severe sepsis.

Methods: Identification and antibiotic sensitivity testing of the GNB isolated from the clinical samples were done using the VITEK-II system in a tertiary care hospital, Kolkata. MDR and XDR strains were selected by their definitions and molecular characterization was done by multiplex polymerase chain reaction. The minimum inhibitory concentration (MIC) value of arbekacin was detected by the E-test strip and compared with other aminoglycosides.

Results: A total of 140 drug-resistant strains including ESBL- and carbapenemase-producing GNB were selected for the study. Arbekacin showed reduced values of MIC_{50} and MIC_{90} compared to other aminoglycosides for most of the drug-resistant GNB.

Conclusion: Hence, in this drug-resistant era, arbekacin with the advantage of a single daily dose can be used as an empirical choice in severe sepsis as monotherapy or in combination with other antibiotics such as colistin or polymyxin to fight against MDR and XDR bugs.

Keywords: Aminoglycoside, Arbekacin, Gram-negative bacilli, Multidrug resistant, Extensively drug resistant.

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INTRODUCTION

Antimicrobial resistance poses a major threat to public health and makes therapeutic decisions more challenging. India carries one of the largest burdens of drug-resistant pathogens worldwide [1]. Emergence of their multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative pathogens adds on to the complexity of infection, rendering them difficult to treat [2]. As there are not enough new antibiotics in the pipeline to meet the challenge of antibiotic resistance, the pharmaceutical industry suggests drug repurposing, to find new uses of old drugs [3]. The drug arbekacin had been licensed in Japan for use under the trade name "Habekacin" for the treatment of MRSA caused sepsis and pneumonia since 1990 [4,5]. Arbekacin is an aminoglycoside dideoxykanamycin B (dibekacin) belonging to the kanamycin family [6]. Arbekacin has the structure of 1-N-[(S)-4-amino-2-hydroxybutyryl (AHB)]-DKB. Due to the introduction of AHB to 1-N position of DKB, the resulting arbekacin exhibited activity against drug-resistant bacteria [7]. Arbekacin is stable to the aminoglycoside-inactivating enzymes such as aminoglycoside phosphotransferase (APH), aminoglycoside adenylyltransferase (AAD), and aminoglycoside acetyltransferase, whereas gentamicin, amikacin, tobramycin, and kanamycin were completely inactivated by APH (2″) [8]. Arbekacin is a good bactericidal drug against MRSA, enterococci and shows concentration-dependent and long-lasting post-antibiotic effects [9-12], but its activity against MDR and XDR Gram-negative bacilli (GNB) is not evaluated. Hence, the objective of the study was to see the *in vitro* activity of arbekacin against MDR and XDR GNB isolated from different clinical samples.

METHODS

Identification of the isolates from clinical samples

The study was conducted in the Department of Microbiology, NRS Medical College, Kolkata, from May 2019 to August 2019. Isolation of GNB was done from different samples (urine, respiratory samples, pus, and blood) by standard microbiological procedure and identification up to the species level was done using VITEK-GN cards (bioMérieux India Private Limited).

Antimicrobial susceptibility testing

Antibiotic sensitivity testing was performed in the VITEK-2 system using AST-GN280 and AST-GN281 susceptibility cards and interpreted according to the Clinical and Laboratory Standards Institute recommendations. *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as quality control strains.

Inclusion and exclusion criteria

Selection for MDR and XDR strains from the isolated GNB was based on the definition of MDR (acquired non-susceptibility to at least one agent in three or more antimicrobial categories) and XDR [non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two antimicrobial categories)]. Isolated GNB strains sensitive or resistant to one/two antimicrobial categories were excluded from the study.

Molecular characterization of MDR and XDR Gram-negative strains

The presence of carbapenemase-encoding genes from the selected MDR and XDR GNB was determined by multiplex PCR using primers (ReadyMade™ *Primers*, Integrated DNA Technologies) (Table 1) targeting *bla*VIM, *bla*IMP, *bla*KPC, *bla*OXA-48, and *bla*NDM (Fig. 1).

ESBL production was determined by placing ceftazidime (CAZ 30 µg) disks with or without clavulanic acid (CA 10 µg) on MHA plate. After overnight incubation, if there was an augmentation of ≥5 mm in the inhibitory zone diameter of CAZ-CA in comparison to CAZ alone, ESBL by that strain was phenotypically confirmed.

The minimum inhibitory concentration (MIC) of arbekacin against MDR and XDR Gram-negative bacterial isolates

To calculate the MIC of arbekacin by E-test strip, a lawn culture is made by the organism to be tested, over a Mueller-Hinton agar plate and the arbekacin E-test strip was applied onto it. After overnight incubation, the reading of MIC was taken as the value at the point where ellipse intersects the scale (Fig. 2).

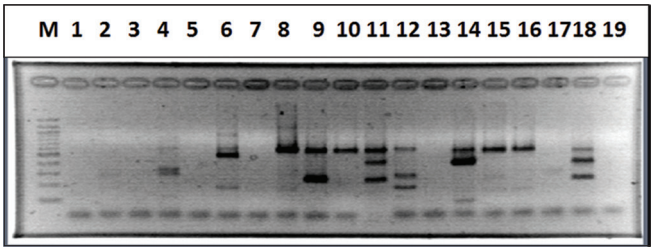

Fig. 1: Multiplex PCR for the detection of carbapenemase-producing genes. M, 100 bp DNA ladder lane: 6, 8, 10, 15, 16 NDM positive (603 bp) Lane: 9, 12 NDM+ OXA-48 (265 bp) positive bacteria. Lane:11, 18 positive control. Lane: 19 negative control

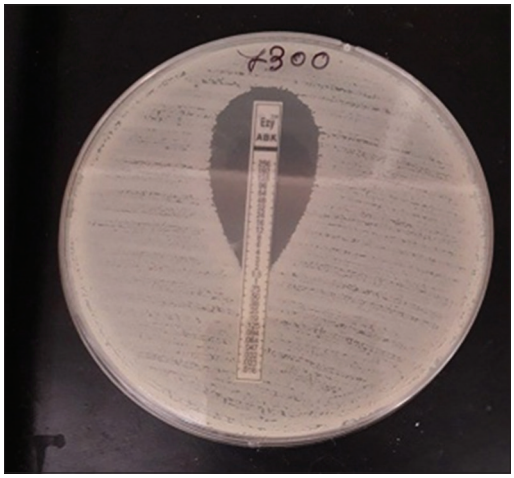

Fig. 2: Arbekacin E-test

RESULTS

Identification of the isolates from clinical samples

A total of 1276 GNB were isolated during the study period. Of them, 526 were *E. coli*, 314 were *Klebsiella pneumoniae*, 24 were *Enterobacter cloacae*, 48 were *Proteus* spp., 190 were *P. aeruginosa*, and 174 were *Acinetobacter* spp.

Among them, 140 representatives drug-resistant GNB were selected according to their antibiotic resistance pattern (Table 2). Of them, 30 were *E. coli*, 55 were *K. pneumoniae*, 12 were *E. cloacae*, 3 were *Proteus* spp., 24 were *P. aeruginosa*, and 16 were *Acinetobacter* spp. Arbekacin breakpoints were used according to Lee *et al*., 2007 [13], as susceptible ≤4 µg/mL; intermediate 8 µg/mL; and resistant ≥16 µg/mL.

Antimicrobial susceptibility testing

Arbekacin showed 50% resistance to *E. coli*, 54.55% resistance to *Klebsiella* spp., 33.34% resistance to *E. cloacae*, 66.67% resistance to *Proteus* spp., 20% resistance to *Pseudomonas* spp., and 50% resistance to *Acinetobacter* spp. (Table 3).

According to the resistance pattern of the selected Gram-negative isolates for the study, 50 strains are MDR and 90 strains are XDR (Table 4).

Among 50 MDR Gram-negative strains, 34 (68%) are sensitive and 16 (32%) are resistant to arbekacin, whereas among 90 XDR Gram-negative strains, 43 (47.78%) are sensitive to arbekacin and 47 (52.23%) are resistant.

The MIC of arbekacin in MDR and XDR Gram-negative strains

MIC_{50} and MIC_{90} of arbekacin for *E. coli* were 1.5 and 16, for *K. pneumoniae* 16 and 64, for *E. cloacae* 0.75 and 8, for *Proteus* spp. 24 and >128, for *Pseudomonas aeruginosa* 1.5 and 8, and *Acinetobacter* spp. 4 and 64, respectively (Table 5).

Table 1: Primer sequences and amplicon sizes

PCR name	Targeted gene	Primer sequence (5′to3′)	Amplicon size
CARBA	blaNDM	Forward ACT TGG CCT TGC TGT CCT T Reverse CAT TAG CCG CTG CAT TGA T	603 bp
	blaVIM	Forward TGT CCG TGA TGG TGA TGA G T Reverse ATT CAG CCA GAT CGG CAT C	437 bp
	blaIMP	Forward ACA YGG YTT RGT DGT KCT TG Reverse GGT TTA AYA AAR CAA CCA CC	387 bp
	blaKPC	Forward TCG CCG TCT AGT TCT GCT GTC TTG Reverse ACA GCT CCG CCA CCG TCA T	353 bp
	blaOXA-48	Forward ATG CGT GTA TTA GCC TTA TCG Reverse CAT CCT TAA CCA CGC CCA AAT C	265 bp
OXA CARBA	blaOXA-23group	Forward CCC CGA GTC AGA TTG TTC AAG G Reverse TAC GTC GCG CAA GTT CCT GA	330 bp

PCR: Polymerase chain reaction, CARBA: Carbapenemase, NDM: New Delhi metallo-β-lactamase, VIM: Verona integron-encoded metallo-β-lactamase, IMP: Imipenemase, KPC: Klebsiella pneumoniae carbapenemase, OXA: Oxacillinase

Table 2: Isolation and identification of selected MDR and XDR GNB from different sources

	Urine	Wound swab	Blood	Sputum and endotracheal aspirates	Total
Escherichia coli	10	-	20	-	30
Klebsiella pneumoniae	30	5	15	5	55
Enterobacter cloacae	10	2	-	-	12
Proteus spp.	1	2	-	-	3
Pseudomonas aeruginosa	5	9	5	5	24
Acinetobacter baumannii	-	5	-	11	16
Total	56	23	40	21	140

Table 3: Resistance (%) pattern of the isolates selected for the study

	AMC	3rd gen Ceph	4th gen Ceph	CFS	PTZ	CIP	LE	ABK	AK	GEN	ETP	IPM	MRP	PB	COT	TGC
Escherichia coli (30)	93.34%	90%	86.67%	83.34%	83.34%	100%	76.67%	50%	83.34%	83.34%	90%	58.34%	58.34%	0%	76.67%	0%
Klebsiella pneumoniae (55)	100%	100%	96.36%	90.90%	81.82%	100%	100%	54.55%	72.73%	81.82%	91%	50%	54.54%	0%	81.82%	0%
Enterobacter cloacae (12)	100%	100%	100%	100%	100%	100%	83.34%	33.34%	66.64%	100%	100%	50%	66.67%	0%	83.34%	0%
Proteus spp. (3)	100%	100%	100%	100%	100%	100%	100%	66.67%	66.67%	66.67%	100%	100%	100%	100%	100%	0%
Pseudomonas aeruginosa (24)	-	100%	100%	83.34%	40%	80%	80%	20%	62.5%	62.5%	-	60%	60%	0%	-	-
Acinetobacter baumannii (16)	-	100%	100%	100%	100%	100%	75%	50%	100%	100%	100%	100%	100%	0%	100%	0%

AMC: Amoxi-clavulanic acid, 3rd gen Ceph: 3rd generation cephalosporin, 4th gen Ceph: 4th generation cephalosporin, CFS: Cefoperazone-sulbactam, PTZ: Piperacillin-tazobactam, CIP: Ciprofloxacin, LE: Levofloxacin, ABK: Arbekacin, AK: Amikacin, GEN: Gentamicin, ETP: Ertapenem, IPM: Imipenem, MRP: Meropenem, PB: Polymyxin B, COT: Cotrimoxazole, TGC: Tigecycline

DISCUSSION

The increased prevalence of MDR and XDR GNB along with their rapid spread is a matter of concern in modern medicine. In our study period over 4 months, we have selected 140 drug-resistant strains including ESBL and carbapenemase-producing GNB which were representative of MDR and XDR strains among the total of 1276 isolates. We compared the resistance pattern of arbekacin with other aminoglycosides (amikacin and gentamicin) and other potent antimicrobial drugs with these selected isolates. Arbekacin showed a better sensitivity profile than penicillin, cephalosporin, other aminoglycosides, fluoroquinolones, β-lactam-β -lactamase inhibitors, and carbapenems. Polymyxin B and tigecycline showed better sensitivity than arbekacin. In *Proteus* spp., the sensitivity of arbekacin was comparable with other aminoglycosides.

This study showed that MIC_{50} of arbekacin was 10.67 times lower than that of amikacin and gentamicin for *E. coli*, 4 times lower than that of amikacin, and 2 times lower than gentamicin in *Klebsiella* spp., 1.33 times lower than that of amikacin, and 2.67 times lower than that of gentamicin for *Enterobacter* spp., 1.33 times lower than that of amikacin but MIC_{50} of gentamicin was 3 times lower than that of arbekacin in *Proteus* spp. In the case of *Pseudomonas* spp., MIC_{50} of arbekacin is 5.33 times lower than that of amikacin and gentamicin and in *Acinetobacter* spp., arbekacin MIC_{50} was 8 times lower than amikacin and 4 times lower than gentamicin. However, the value of MIC of arbekacin was high in *Klebsiella* (MIC_{50} = 16, MIC_{90} ≥ 64) and *Proteus* (MIC_{50} = 24, MIC_{90} ≥ 128) (Table 5). In these cases, we might consider the combination therapy with polymyxins/tigecycline or carbapenem (if carbapenem was sensitive) [14]. Antibiotic combination therapy study groups suggested the effective combination of arbekacin plus aztreonam against MDR *Pseudomonas* [15].

Hence, the MIC value of arbekacin is lower than other aminoglycosides in drug-resistant GNB which was similar to the study by Watanabe *et al*., 2012, Kazuno *et al*., 1986 [16,17]. Arbekacin is a unique aminoglycoside because of its ability to concentrate in pulmonary epithelial lining fluid [18], unlike other aminoglycosides which will help us to use it against pneumonia and other respiratory diseases. Again, simultaneous Gram-positive and Gram-negative coverage with a single daily dose are the advantage of arbekacin and it can be used as an empirical choice in severe sepsis. To interpret its *in vitro* activity, the sensitivity of other aminoglycosides (amikacin and gentamicin) can guide arbekacin sensitivity for treating physicians in a resource-limited setting. Although the sensitivity of tigecycline is better than arbekacin, the favorable pharmacodynamics of arbekacin make the drug superior to tigecycline.

A higher dose of arbekacin can improve clinical efficacy. In a study by Matsumoto *et al*., 2014 [8], it was shown that in patients with normal renal function, the target peak concentration (Cpeak) was not achieved with once-daily administration of 150–200 mg and a higher dosing regimen is required to improve clinical efficacy, and the Japanese Society of Chemotherapy and the Japanese Society of Therapeutic Drug Monitoring Experts recommend 5.5–6.0 mg/kg body weight to reach the target concentration [19]. The pharmacokinetics in healthy volunteers with normal renal function did not change on 400 and 600 mg single dose and the total clearance does not decrease at a high dose [8].

Table 4: Number of MDR and XDR strains selected for the study

	MDR strains	XDR strains
Escherichia coli	10	20
Klebsiella pneumoniae	20	35
Enterobacter cloacae	9	3
Proteus spp.	0	3
Pseudomonas aeruginosa	11	13
Acinetobacter baumannii	0	16

MDR: Multidrug resistant, XDR: Extensively drug resistant

Table 5: Comparison of MIC of arbekacin with gentamicin and amikacin against selected drug resistant Gram-negative isolates

Isolates	Molecular characterization of the isolates	Arbekacin MIC (µg/ml)		Amikacin MIC (µg/ml)		Gentamicin MIC (µg/ml)		Range of MIC in arbekacin (µg/ml)
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	
Escherichia coli (n=30)	21 *E. coli* are ESBL producing and 16 *E. coli* are NDM producing	1.5	16	16	>64	16	>64	0.19–>32
Klebsiella pneumoniae (n=55)	8 are NDM+OXA48 producing, 19 are NDM producing and 38 are ESBL producing	16	>64	64	>128	32	>128	1–>128
Enterobacter cloacae (n=12)	6 are ESBL producing	0.75	8	1	32	2	64	0.25–1
Proteus spp. (n=3)	3 are ESBL producing	24	>128	32	>128	8	>128	8–>128
Pseudomonas aeruginosa (n=24)	9 are ESBL producing	1.5	8	8	64	8	>64	0.19–16
Acinetobacter baumannii (n=16)	7 are ESBL producing and 9 are NDM+OXA23 producing	4	64	32	>128	16	>128	0.25–>64

MIC: Minimum inhibitory concentration

If the dose of arbekacin is formulated as 4 times the normal dose with dose spacing of 72 h, it will have the following benefits: (i) The dose will cross 4 times the normal MIC which is well above minimum bactericidal concentration (MBC) and for that the chance of cross-resistance or resistant mutant will be nil. (ii) If the dose is 4 times increased, it will be far less than the toxic dose and therefore safe. The renal-related adverse drug reactions of arbekacin are increased with a higher Ctrough [11]. The incidence of arbekacin induced nephrotoxicity was observed when it was administrated at a total dose of over 5000 mg [20]. Moreover, with a dose spacing of 72 h, the Ctrough will be lower. (iii) Outpatient antimicrobial therapy is possible in resource-limited settings. (iv) It can be cost effective and can be used in remote places where a basic sensitivity pattern is available.

Hence, in case of severe sepsis, we can plan the treatment in the following way – first, send the culture from all relevant sites and give arbekacin 800 mg (4 times the normal dose to attend the MBC). No antibiotics are needed for the next 72 h until the culture sensitivity report comes and plan the antibiotics accordingly. All other supportive measures are to be continued as per the sepsis protocol. The total cost of therapy will be less with a fair chance of patient survival.

CONCLUSION

Hence, in this drug-resistant era, arbekacin can be used as an empirical choice in severe sepsis as monotherapy or in combination with other antibiotics such as colistin or polymyxin to fight against MDR and XDR bugs. The favorable pharmacokinetics, pharmacodynamics, and spectrum (simultaneous Gram-positive and Gram-negative coverage) of the drug with the advantage of a single daily dose will make the antibiotic as a handy choice for the management of sepsis with least time to the thermometer to needle even in resource-limited health-care settings in India.

AUTHORS' CONTRIBUTIONS

Dr. Soma Sarkar – Data collection and manuscript writing, Dr. Dipankar Sarkar – Design the research study, Dr. Anjum Namhata – Preparation of manuscript, and Dr. Manideepa Sengupta – Editing of the manuscript as per journal guidelines.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS' FUNDING

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