ORIGINAL ARTICLE=

In-Vitro Efficacy of Imipenem with Tigecycline and Amikacin against Extensively Drug Resistant Acinetobacter Baumannii

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ABSTRACT

Aim: To find out effective antibiotic combinations against extensively drug resistant *Acinetobacterbaumannii* due to emerging issues of treatment failures.
Design: It was a descriptive study.
Place and duration: Department of Microbiology University of Health Sciences Lahore from 1st March 2017 to 31st March 2018.
Methodology: Total 50 clinical isolates were collected and identified by standard microbiological procedures including Kirby Bauer disc diffusion for antibiotic susceptibility. Agar dilution method was used to determine the Minimal inhibitory concentration (MIC) for the antibiotics according to Clinical and Laboratory Standards of Institute, CLSI 2017 guidelines. Microdilution checkerboard titration technique was performed to determine the synergistic combinations of antimicrobials by calculating fractional inhibitory concentrations (FICs).
Results: In-vitro indifferences among imipenem-tigecycline 49(98%) and imipenem-amikacin 34(68%) combinations was found. Antagonism among combinations of imipenem-tigecycline 1(2%) and imipenem-amikacin 16(32%) was noted.
Conclusion: It is concluded from the study that combination of imipenem with tigecycline or amikacin is not a valuable option for treatment against extensively drug resistant *Acinetobacterbaumannii*.
Keywords: *Acinetobacterbaumannii*, Antibiotic susceptibility, Minimum inhibitory concentration, Microdilution checkerboard titration technique, Fractional inhibitory concentrations, Antagonism.

INTRODUCTION

The genus Acinetobacter is a cluster of ubiquitous microorganisms and is found in the surroundings[1]. *Acinetobacterbaumannii* has gained no importance until 1970 and considered as a low grade pathogen[2]. It has been currently evolved as one of the most difficult hospital acquired pathogen to treat across the globe and responsible for many hospitals and community acquired infections[3]. Treatment of extensively drug resistant *Acinetobacterbaumannii* infections is challenging. The prolonged survival ability of this notorious microorganism in any environment is the main reason for its transmission in health care settings[4]. It is the major reason behind wide spectrum of diseases which include wound infections, pneumonia, endocarditis, urinary tract infections etc[5]. *A.baumannii* has been approved for its biofilm forming ability that is believed to play an important role in the process of colonization[6]. *A.baumannii* was responsive to many classes of antibiotics till early 1970[7]. However, since 1975 *A.baumannii* resistance was developed against all classes of antibiotics[8]. From the year 2000 to date, different antibiotic combinations have been evaluated for their synergistic activity or to treat this highly resistant pathogen[9,10,11,12,13,14].

The focus of this study was to determine a synergistic combination of imipenem with either of the antibiotics tigecycline and amikacin by a checkerboard microtitration technique.

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MATERIALS AND METHODS

This descriptive study was conducted in Microbiology Department University of Health Sciences Lahore, Pakistan over a year of one year from 1st March 2017 to 31st March 2018 approved by ethical review committee of University of Health Sciences. Total 50 samples of *A.baumannii* were collected within three months from February to March 2017 from Services Hospital Lahore. These samples were collected in tryptic soya agar slopes.

Bacterial strains identification: The extensively drug resistant *A.baumannii* (XDR-AB) phenotype was identified and included in the study while multidrug resistant and Pan drug resistant *A.baumannii* were excluded.The identification was done bysubculturing and purifiying the organism on tryptic soya and MacConkeys agar plates, biochemical reactions and finally by using API 20-NE (BioMerieux, France).The strains selected were stored in microbanks at a temperature of -80ºC.

Antimicrobial Susceptibility Testing: These strains were resistant to all classes of antibiotics except tigecycline and doxycycline by using Kirby Bauer disc diffusion method recommended by Clinical and Laboratory Standards Institute (CLSI,2017). For tigecycline Food Development Administration (FDA) criteria for zone of diameter for Enterobacteriaceae was used as no guideline is given in CLSI 2017.

Antibiotic agents and minimal inhibitory concentration determination: Imipenem (Bosch Pharmaceuticals), tigecycline (WYETH PAKISTAN) and amikacin (Sami Pharmaceuticals) were used as base materials. Stock solutions of antibiotics were prepared according to CLSI 2017 guidelines in their respective solvents (Phosphate buffer for imipenem and water for tigecycline and amikacin). These stock solutions were stored at -20ºC for one week. Standard agar dilution method was used to

determine MICs for all strains against each antibiotic. Bacterial inoculum equal to 0.5 McFarland ($5x10^8$) was prepared and diluted upto 1:10 to achieve a final concentration of $5x10^7$CFU/ml. For each antibiotic a concentration range was prepared in mullerhinton agar: 0.5to16µg/ml for imipenem and tigecycline and 4to128µg/ml for amikacin. With multipoint inoculator(MAST Diagnostics UK) bacterial suspension was inoculated on the agar plates and incubated at 37ºC for 24 hrs. After incubation the lowest concentration noted at which growth of the organism was inhibited and MIC breakpoints were determined (CLSI 2017). MIC breakpoints for tigecycline was determined by FDA. *Escherichia coli* ATCC 25922 and *Acinetobacterbaumannii* ATCC 19606 were used as controls.

Synergy Testing: Microdilution checkerboard titration method was used for synergy testing of antibiotics. Concentration range of antibiotic was choosen according to the already determined MIC for each isolate. The following formula was used for calculation of FICs and FICI for each antibiotic.

ΣFIC or FICI=FIC A+ FIC B
Where;
FIC A= MIC of drug A in combination / MIC of drug A alone
And

FIC B= MIC of drug B in combination / MIC of drug B alone

When ΣFIC is ≤ 0.5 it will be synergistic, ≤0.5 and≤4.0 it will be indifferent and antagonistic when ΣFIC will be > 4.0[12].

Data analysis: Data analyzed by using SPSS 20.For descriptive statistics results were presented in the form of percentages or frequencies. Fractional inhibitory concentrations were expressed by its formula by adding drug A and drug B.

RESULTS

Sources from which *A. baumannii*was isolated has been shown in Figure I. Central venous catheter tips 24(48%) contains the highest number of *A.baumannii* followed by pus 20(40%), high vaginal swab 2(4%),urine 2(4%) and fluid 2(4%). All isolates were resistant to all classes of antibiotics except tigecycline (100% sensitive) and doxycycline (62% sensitive).

MIC_{90} for tigecycline,imipenem and amikacinwas 2 , 16 and 64µg/ml respectively. Their MIC ranges and sensitivity rate has been shown in Table I. MIC for ATCC *E.coli* and *A.baumannii* strains are also shown in table I. MICs of XDR *A.baumannii* for three antibiotics by broth microdilution method and Results of microtitration checkerboard method are shown in Table II. Sixteen isolates 16(32%) AB-03,AB-05,AB-06,AB-09,AB-11,AB-12,AB-13,AB-14,AB-15,AB-28,AB-30,AB-31,AB-34,AB-36, AB-37andAB-48 showed antagonistic effect in imipenem-amikacin combination while 34(68%) showed indifference. In case of above mentioned isolates, MIC of imipenem in combination was increased four to eight times than the individual MIC against these isolates. Synergism was not detected in this combination. In case of imipenem-tigecycline combination one strain, 1(2%) AB-44 showed antagonistic effect with four times increased MIC in combination. Rest of the isolates 49(98%) showed indifference with no synergism in this combination as well.

Fig. I. Sources of XDR *A. baumannii* isolates

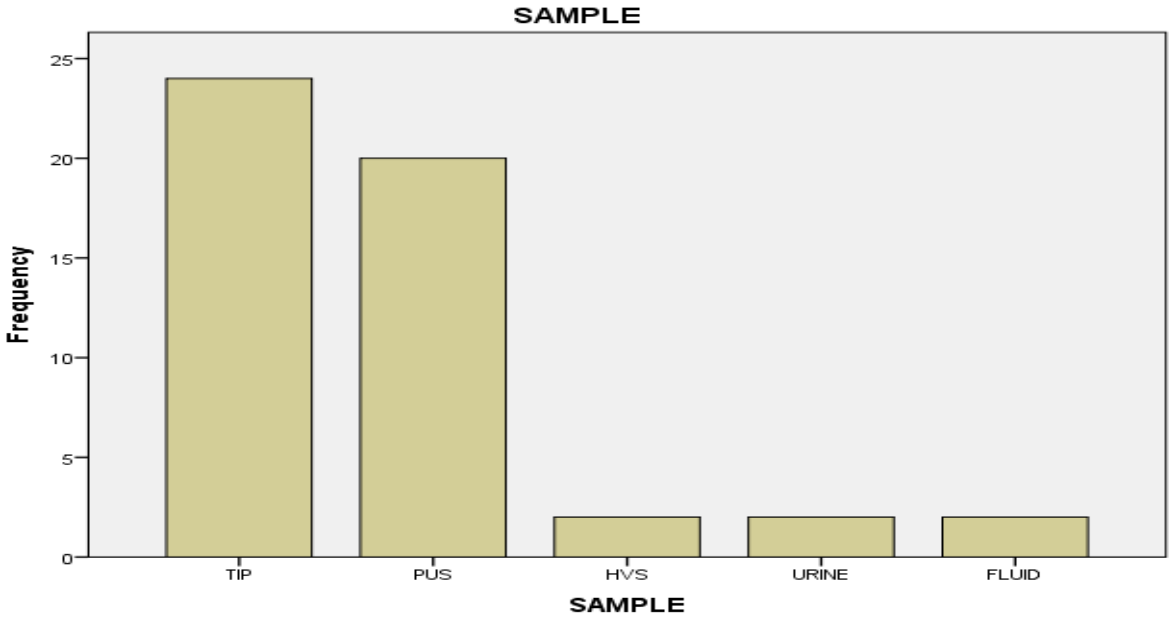

Table I. Minimal inhibitory concentration(MICs),sensitivity rates and quality control ranges of XDR *A.baumannii*(n=50) and *E.coli* ATCC 25922 against 3 different antibiotics

Antibiotic	XDR *A.baumannii*(n=50)					*E.coli* ATCC [a]25922(n=1)	
	MIC(µg/ml)		Sensitivity Rate(%)[b]			MIC µg/ml	MIC QC Ranges[d]
Range[c]	MIC_{50}	MIC_{90}	S	I	R	µg/ml	
Imipenem	0.5-16	4.0	16.0		100	0.25	0.06-0.25
Tigecycline	0.5-16	2.0	2.0	100		0.25	0.03-0.25
Amikacin	4.0-128	16.0	64.0	34	66	1.0	0.5-4.0

a; American Type Culture Collection, b; S=sensitive, I= intermediate, R= resistant interpreted by Clinical Laboratory Standard (CLSI) 2017 guidelines, c; Sensitivity range given by CLSI 2017 guidelines, d; Quality control ranges provided by CLSI 2017 guidelines against ATCC reference strain.

Table II. Minimal inhibitory concentrations (MICs) of XDR *A.baumannii*(n=50) against 3 different antibiotics by broth microdilution method and Test results of antibiotic combination by checkerboard microtitration method.

ISOLATE NO.	MICµg/ml Imipenem	MICµg/ml Tigecycline	MICµg/ml Amikacin	IMP/TGC	Σ FIC	IMP/AK	Σ FIC
AB-01	2.0	2.0	64.0	1.0	ID	1.03	ID
AB-02	2.0	2.0	64.0	2.5	ID	2.03	ID
AB-03	4.0	2.0	16.0	1.5	ID	4.5	AG
AB-04	4.0	2.0	16.0	1.0	ID	2.25	ID
AB-05	4.0	2.0	8.0	1.5	ID	8.25	AG
AB-06	0.5	2.0	8.0	1.0	ID	4.25	AG
AB-07	16.0	2.0	64.0	1.5	ID	2.25	ID
AB-08	1.0	2.0	64.0	1.0	ID	0.75	ID
AB-09	16.0	2.0	4.0	2.5	ID	4.25	AG
AB-10	16.0	2.0	4.0	2.5	ID	1.25	ID
AB-11	0.5	2.0	16.0	1.5	ID	4.25	AG
AB-12	0.5	2.0	64.0	2.5	ID	4.25	AG
AB-13	16.0	2.0	64.0	1.0	ID	4.25	AG
AB-14	2.0	2.0	8.0	2.5	ID	4.25	AG
AB-15	2.0	2.0	8.0	2.5	ID	4.25	AG
AB-16	16.0	2.0	16.0	1.0	ID	1.25	ID
AB-17	16.0	2.0	64.0	2.5	ID	0.75	ID
AB-18	1.0	2.0	64.0	1.0	ID	0.75	ID
AB-19	1.0	2.0	64.0	1.5	ID	2.25	ID
AB-20	16.0	2.0	4.0	1.0	ID	0.75	ID
AB-21	16.0	2.0	4.0	1.5	ID	1.25	ID
AB-22	16.0	2.0	16.0	1.0	ID	0.75	ID
AB-23	16.0	2.0	64.0	2.5	ID	2.25	ID
AB-24	0.5	2.0	8.0	2.5	ID	1.25	ID
AB-25	0.5	2.0	8.0	2.5	ID	0.75	ID
AB-26	1.0	2.0	64.0	2.5	ID	1.25	ID
AB-27	1.0	2.0	64.0	1.5	ID	1.25	ID
AB-28	16.0	2.0	64.0	1.0	ID	4.25	AG
AB-29	16.0	2.0	64.0	1.5	ID	2.25	ID
AB-30	16.0	2.0	64.0	1.0	ID	4.25	AG
AB-31	2.0	2.0	64.0	1.0	ID	4.25	AG
AB-32	2.0	2.0	64.0	1.0	ID	2.25	ID
AB-33	16.0	2.0	64.0	1.5	ID	0.75	ID
AB-34	16.0	2.0	16.0	1.5	ID	4.25	AG
AB-35	4.0	2.0	4.0	2.5	ID	1.25	ID
AB-36	4.0	2.0	16.0	2.5	ID	4.03	AG
AB-37	16.0	2.0	64.0	1.5	ID	8.03	AG
AB-38	16.0	2.0	64.0	2.5	ID	2.03	ID
AB-39	16.0	2.0	64.0	1.0	ID	1.03	ID
AB-40	0.5	2.0	8.0	2.5	ID	2.03	ID
AB-41	0.5	2.0	8.0	1.5	ID	1.03	ID
AB-42	16.0	2.0	16.0	1.5	ID	2.03	ID
AB-43	16.0	2.0	64.0	2.5	ID	1.03	ID
AB-44	16.0	2.0	64.0	4.5	AG	2.03	ID
AB-45	16.0	2.0	64.0	1.5	ID	2.03	ID
AB-46	16.0	2.0	64.0	1.5	ID	1.03	ID
AB-47	16.0	2.0	16.0	1.5	ID	1.03	ID
AB-48	16.0	2.0	16.0	2.5	ID	4.03	AG
AB-49	2.0	2.0	8.0	1.5	ID	1.03	ID
AB-50	2.0	2.0	8.0	1.0	ID	2.03	ID

DISCUSSION

A.baumannii isdiffssicult to treat pathogen and has become a great challenge for the physicians[4]. The presence of MDR and XDR *A.baumannii* has become a global threat as more cases of morbidity and mortality are noted[15,16]. Different antibiotic combinations have been used in past and recently to find out synergism in vitro or in vivo against *A.baumanni*[17,18,19,20,21]. Increased antibiotic resistance against *A.baumannii* has been reported[22]. Our study showed 100% susceptibility of XDR *A.baumannii* to tigecycline but due to high mortality rate by using tigecycline as a mono-therapeutic drug in severe bloodstream infections combination therapy with different antibiotics is required[23]. Among different studies tigecycline has shown great sensitivity of 92%, 99% and 99.3% that is close to our study as well[24,25,26]. All strains were resistant to imipenem 100% and amikacin 66% in our study. Increased resistance toimipenem and amikacin has been reported in various regions of the world. A study done in Taiwan from the year 2002 to 2010 has reported resistance increased from 63.1% to 64.3% in case of amikacin and 3.4% to 58.7% in imepenem among XDR strains of *A.baumannii*[27]. Another study done in Beijing from the year of 2004 to 2014 showed increased resistance to imipenem and amikacin from a rate of 13.3% to 70.5% and 56.2% to 66% respectively[28]. In this study XDR isolates of *A.baumannii* were selected because they have become a major problem and difficult to treat in our setup. This study showed 98% indifference and 2% antagonism in the combination of imipenem and tigecycline while 68% indifference and 32% antagonism for the combination of imipenem and amikacin with no synergism at all. A study reported 85.7% indifference, 8.3% antagonism and only 5.3% synergism of tigecycline with different antibiotics among MDR strains[19]. Another study showed 30% antagonistic effect between imipenem and tigecyclineamong MDR strains of *A.baumannii*[29]. As our study choose XDR strains of *A.baumannii* with different antibiotic combinations, methodology, and resistance patterns of the bacterial strains so that could be a possible reason for different results from above mentioned studies and reduced efficacy of the drug due to its interaction with other drug could be another cause[30].

CONCLUSION

From our study data it is concluded that imipenem is not effective in combination with tigecycline or amikacin however tigecycline alone or in combination with other antibiotics has shown excellent results but clinical implication on patients is limited so is the data hence needs improvement at both hands.The limitations to our study is that the samples were collected from a single hospital and genotypic analysis should be done to rule out the resistance patterns of each strain. More antibiotic combinations should be tested in order to find a valuable option against XDR *A. baumannii*.

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Conflict of Interest: There is no conflict of interest among the authors

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