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Efficacy of Antibiotic Combinations on Multi-Drug Resistant Bacterial Strains Isolated from Urinary Tract Infection and Hemodialysis Patients

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Abstract: Urinary tract infection (UTI) is the second most common type of infections in the body and it affects millions of people each year. The increasing rate of antimicrobial agents' consumption to treat infections has resulted in emergence of resistance even to more potent antibiotics. A total of 12 bacterial clinical isolates were collected from chronic hemodialysis (one isolate) and out-patients of UTI (11 isolates) of International hospital for Urology and Nephrology, El- Giza, Egypt, during 7/2013:12/2013. The clinical isolates have been investigated against 20 different antibiotics. Eleven of the clinical isolates out of 12 showed resistance against all tested antibiotics and the other isolate showed resistance against 19 tested antibiotics. The isolates were resistant to imipenem (IMP), amikacin (AK), cefepime (FEP) and tigecycline (TIG) antibiotics alone or in combinations. The combination (AK/IMP/TIG/FEP) was the only one which achieved > 90% killing against all of the isolates. The three combinations (AK/TIG/IMP, TIG/IMP/FEP and TIG/AK/FEP) also achieved > 90% killing against the isolate *Staphylococcus* sp. (1F).

Keywords: Urinary tract infection, Multidrug resistance, hemodialysis, antibiotic combinations.

1 Introduction

Urinary tract infection (UTI) is one of the most common infectious diseases in the world [1]. The resistance of uropathogens to commonly used antimicrobial agents is increased worldwide [2]. Increased consumption of antibiotics to treat human infections leads to the selection of multi-drug resistant (MDR) pathogens which cause problems in treating patients [3]. Over the past 40 years, there was little novel classes of antibiotics were created which resulted in increasing incidence of infections caused by MDR pathogens which becomes a global health problem [4]. MDR pathogens are resistant to most if not all of the commonly used antibiotics as they have many resistance mechanisms [5].

MDR nosocomial infections became a concerned health crisis [6]. The increasing prevalence of MDR uropathogens, including *E-coli*, which cause most of UTIs, is a worldwide problem [7]. Extended spectrum β lactemases-producing *Enterobacteriaceae* are often MDR uropathogens [8]. The prevalence of drug resistance of uropathogenic *E-coli* to fluoroquinolones, cephalosporins, penicillins, and trimethoprim-sulfamethoxazole has decreased antimicrobial agents' treatment options [9].

Using two antimicrobial agents in a combination have a stronger effect than using one drug alone [10]. Antibiotics combinations are increasingly used to increase the antimicrobial effects of commonly used antimicrobial agents against MDR pathogens [11]. As antibiotic combinations have different mechanisms of action against those pathogens [12]. Severe Gram-negative infections are often treated with antibiotics combinations but that is debatable [11]. Antibiotic selection for treating infections should be based on knowledge of resistance risk factors including previous infection with resistant pathogens, hospitalization and recent antimicrobial use besides the local resistance epidemiology [13].

Antibiotic combinations may cause bactericidal or bacteriostatic effects which have a major concern in treating infections [14]. Using Antimicrobial combinations may reduce the risk of emerging resistance during therapy, give a broader antibacterial spectrum and have synergistic effects [11]. Synergism is an interaction in which antimicrobial drugs effects have been increased when used in a combination [10]. Therefore, studies should be taken seriously to produce novel antimicrobial combinations [15]. The aim of the present study was to investigate the efficacy of antibiotic combinations on MDR pathogens in Egypt.

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2 Materials and Methods

2.1 Isolation and Identification of clinical human bacterial pathogens

The clinical samples were collected from chronic hemodialysis and out-patients of UTI of International hospital for urology & nephrology (private hospital in El-Giza, Egypt). The collected samples were streaked on the surface of L.B agar plates [16], Blood agar, McConkey agar medium [17] and Cystine-lactose-electrolyte deficient (CLED) [18] using sterile standard wire loop under aseptic condition. The inoculated plates were incubated at 37°C for 24 h. Purification of the bacterial isolates was achieved by subculturing on selective media (McConkey, Blood agar and CLED). Identification and confirmation have been achieved according standard laboratory procedures [18] involving Gram stain, rapid tests (catalase, oxidase, coagulase), and biochemical tests (Indole, Citrate, Triple sugar iron, oxidation, fermentation, urease and hemolysin production). Single isolate was selected from each sample.

2.2 Antibiotic Discs

The antibiotic discs which used in the study were purchased from Bioanalyse, Ltd, Ankara, Turkey. The following antibiotic discs were used: Penicillin (P, 10 U); Amoxicillin (AX, 25 µg); Amoxicillin/clavulanic acid (AMC, 20/10 µg); Piperacillin (PRL, 100 µg); Cefaclor (CEC, 30 µg); Cephradine (CE, 30 µg); Aztreonam (ATM, 10 µg); Cefepime (FEP, 30 µg); Cefotaxime (CTX, 30 µg); Trimethoprim (TMP, 5 µg); Pefloxacin (PEF, 5 µg); Sparfloxacin (SPX, 5 µg); Ofloxacin (OFX, 5 µg); Norfloxacin (NOR, 10 µg); Ciprofloxacin (CIP, 5 µg); Gentamicin (CN, 10 µg); Streptomycin (S, 10 µg); Specitinomycin (SPT, 10 µg); Amikacin (AK, 30 µg) and Tobramycin (TOB, 10 µg).

2.3 Antimicrobial susceptibility tests

Antimicrobial susceptibility patterns were determined according to the Clinical and Laboratory Standards Institute (CLSI)-recommended modified Kirby-Bauer disc diffusion method on L.B agar plates with commercial antibiotic discs [19].

A loopful of each clinical isolates was inoculated into 3.0 ml sterile L.B broth medium and adjusted to 10^3 CFU/ml using McFarland standards. About 0.1 ml of each isolate was inoculated on the surface of Mueller-Hinton agar plates [17] and antibiotic discs were placed on the surface using sterile forceps under aseptic condition. All plates were incubated up-right at 37°C for 24 h. and inhibition zone diameter (mm) around each antibiotic disc has been determined. Two replicates were used for each antibiotic and each clinical isolate. Those isolates which showed resistance to at least one antibiotic in three or more antimicrobial classes were considered MDR [20].

2.4 MIC determination

Antibacterial activity in terms of minimum inhibitory concentration (MIC) was determined as described by Banjara *et al.*, [21] using L.B broth dilution method. Twelve MDR pathogenic isolates were selected for MIC determination. The selected isolates were inoculated in L.B broth medium and incubated in shaking incubator (150 rpm) at 37°C for 24 hours. Three antibiotics imipenem (IMP), amikacin (AK) and cefepime (FEP) were purchased from Egyptian pharmacy (Intravenous powder antibiotics). Stock solutions of (50000 µg/ml) have been prepared. Twenty ml of sterilized L.B broth in 100 ml conical flasks were supplemented with a double fold dilution of antibiotic concentrations (0, 2, 4, 8, 16, 32, 64, 128, 256, 512 and 768 µg/ml) for each isolate in duplicates. The flasks were inoculated with 2 ml (1×10^5 CFU/ml) and incubated at 37°C for 18 h. in shaking incubator. Positive control was L.B broth inoculated with bacterial isolates without antibiotic and negative control was L.B broth containing antibiotics without bacterial isolates. The optical density (OD) was determined at 600 nm spectrophotometerically (Spectrophotometer SL27, Elico, Ltd, Telangana, India). Percentage of growth inhibition was calculated as:

$$\text{Percentage of growth inhibition} = \frac{\text{OD control} - \text{OD antibiotic}}{\text{OD control}} \times 100$$

MIC_{50} means the lowest concentration of the antibiotic which results in 50% growth inhibition

MIC_{90} means the lowest concentration of the antibiotic which results in 90% growth inhibition as mentioned by Akujobi and Njoku, [22].

2.5 Determination of antibacterial activity of antibiotic combinations

Antibacterial activity of eleven different antibiotic combinations was also determined as described by Banjara *et al.*, [21] using L.B broth dilution method as described before on the same 12 MDR pathogenic isolates. The selected isolates were inoculated in L.B broth medium and incubated in shaking incubator (150 rpm) at 37°C for 24 hours. Three stock solutions of 50000 µg/ml of IMP, AK, FEP and a stock solution of 5000 µg/ml of tigecycline (TIG), which also purchased from Egyptian pharmacy (Intravenous powder antibiotics), have been prepared. The eleven tested antibiotic combinations were: (1) AK/IMP; (2) AK/FEP; (3) IMP/FEP; (4) AK/TIG; (5) TIG/IMP; (6) TIG/FEP; (7) AK/IMP/FEP; (8) AK/TIG/IMP; (9) TIG/IMP/FEP; (10) AK/TIG/FEP and (11) AK/TIG/IMP/FEP. All experiments were carried out in duplicates.

Twenty ml of sterilized L.B broth in 100 ml conical flasks were supplemented with antibiotics with a double of its resistant breakpoint concentration (16, 64 and 128 µg/ml for

TIG, FEP and AK, respectively), and 64 µg/ml for IMP for each isolate in duplicates. The flasks were inoculated with 2 ml (1×10^5 CFU/ml) and incubated at 37°C for 18 h. in shaking incubator. Positive control was L.B broth inoculated with bacterial isolates without antibiotic and negative control was L.B broth containing antibiotics without bacterial isolates. OD was determined at 600 nm spectrophotometerically after 24 hours and percentage of growth inhibition was also calculated as described before. The combination was considered synergy when it caused ≥ 90% killing by the drug combination [23].

3 Results and Discussion

A total of 12 multi-drug resistant (MDR) bacterial isolates were isolated from samples of urine and blood (11 and 1) respectively. Out of the 12 isolates, Gram-negative bacilli accounted for 75% while Gram-positive cocci accounted for the remaining 25% of the total isolates. The isolated pathogens were 3 *Pseudomonas* spp., 3 *Staphylococcus* spp., 3 *E. coli*, 1 *Acinetobacter* sp., 1 *Klebsiella* sp., and 1 *Proteus* sp.

Antibiotic susceptibility of the 12 clinical isolates was shown in Table (1). The results revealed that all the isolates were MDR pathogens, 11 of them were resistant to the twenty used antibiotics (100%) and one of them (*Klebsiella* sp. 35F) was resistant to 19 antibiotics.

The results also revealed that these isolates were resistant to imipenem, amikacin and cefepime. MIC_{50} values of imipenem, amikacin and cefepime ranged 128 - > 768 µg/ml, 16 - 768 µg/ml and 64 - > 768 µg/ml respectively. The MIC_{90} values of imipenem, amikacin and cefepime ranged > 768 µg/ml, 768 - > 768 µg/ml and > 768 µg/ml respectively against all the twelve isolates as indicated in Table (2).

Percentage of growth inhibition of the twelve MDR pathogenic bacteria by antibiotic combinations were determined as indicated in table (3). The combination (AK/IMP/TIG/FEP) was the only one, which achieved > 90% killing after 24 h exposure against all of the isolates. The three combinations (AK/TIG/IMP, TIG/IMP/FEP and TIG/AK/FEP) also achieved > 90% killing after 24 h exposure against the isolate *Staphylococcus* sp. (1F).

The present study provides the information about the antibiotic resistance pattern of bacterial pathogens isolated from UTI and chronic hemodialysis patients and about the efficacy of antibiotic combinations on multi-drug resistant bacterial strains.

The MIC_{90} values of imipenem (IMP), amikacin (AK) and cefepime (FEP) ranged > 768 µg/ml, 768 - > 768 µg/ml and > 768 µg/ml respectively against all isolates. The results of the present study were confirmed by the results of other investigators as the following:

Aboulmagd and Alsultan, [23] also found that the MIC values of IMP, AK, FEP and TIG ranged 16 - >32 µg/ml, 32 - 128 µg/ml, > 256 µg/ml and 16 - 64 µg/ml respectively against all isolates. Esimone *et al*., [24] found that the MIC of the isolated *S. aureus*, *E. coli*, and *Klebsiella* species to ofloxacin, ciprofloxacin, pefloxacin, and co-trimoxazole were > 500 µg/ml.

Table (1): Antibiotic susceptibility of MDR bacterial isolates against 20 antibiotics

Isolates	Code	P	AX	AMC	PRL	CE	CEC	CTX	FEP	ATM	AK
Pseudomonas sp.	38D	R	R	R	R	R	R	R	R	R	R
Pseudomonas sp.	42D	R	R	R	R	R	R	R	R	R	R
Pseudomonas sp.	50D	R	R	R	R	R	R	R	R	R	R
E. coli	46D	R	R	R	R	R	R	R	R	R	R
E. coli	16F	R	R	R	R	R	R	R	R	R	R
E. coli	39F	R	R	R	R	R	R	R	R	R	R
Staphylococcus sp.	39D	R	R	R	R	R	R	R	R	R	R
Staphylococcus sp.	1F	R	R	R	R	R	R	R	R	R	R
Staphylococcus sp.	38F	R	R	R	R	R	R	R	R	R	R
Acinetobacter sp.	37D	R	R	R	R	R	R	R	R	R	R
Klebsiella sp.	35F	R	R	R	R	R	R	R	R	R	R
Proteus sp.	34D	R	R	R	R	R	R	R	R	R	R
Isolates	**Code**	**TOB**	**CN**	**SPT**	**NOR**	**PEF**	**CIP**	**OFX**	**SPX**	**TMP**	**S**
Pseudomonas sp.	38D	R	R	R	R	R	R	R	R	R	R
Pseudomonas sp.	42D	R	R	R	R	R	R	R	R	R	R
Pseudomonas sp.	50D	R	R	R	R	R	R	R	R	R	R
E. coli	46D	R	R	R	R	R	R	R	R	R	R
E. coli	16F	R	R	R	R	R	R	R	R	R	R
E. coli	39F	R	R	R	R	R	R	R	R	R	R
Staphylococcus sp.	39D	R	R	R	R	R	R	R	R	R	R
Staphylococcus sp.	1F	R	R	R	R	R	R	R	R	R	R
Staphylococcus sp.	38F	R	R	R	R	R	R	R	R	R	R
Acinetobacter sp.	37D	R	R	R	R	R	R	R	R	R	R
Klebsiella sp.	35F	R	R	R	R	R	I	R	R	R	R
Proteus sp.	34D	R	R	R	R	R	R	R	R	R	R

Mueller-Hinton agar (Oxoid) medium meets the CLSI standard M6-A2.

Table (2): MIC_{50} and MIC_{90} of Amikacin, Cefepime and Imipenem

Isolates	Code	Amikacin		Cefepime		Imipenem	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
***Proteus* sp.**	34D	512	> 768	768	> 768	128	> 768
***Acinetobacter* sp.**	37D	512	> 768	128	> 768	768	> 768
***Pseudomonas* sp.**	38D	256	> 768	128	> 768	256	> 768
***Staphylococcus* sp.**	39D	768	> 768	64	> 768	512	> 768
***Pseudomonas* sp.**	42D	256	> 768	256	> 768	256	> 768
E. coli	46D	512	> 768	64	> 768	512	> 768
***Pseudomonas* sp.**	50D	512	> 768	> 768	> 768	> 768	> 768
***Staphylococcus* sp.**	1F	128	768	64	> 768	> 768	> 768
E. coli	16F	256	> 768	512	> 768	768	> 768
***Klebsiella* sp.**	35F	512	> 768	128	> 768	768	> 768
***Staphylococcus* sp.**	38F	16	> 768	768	> 768	768	> 768
E. coli	39F	512	> 768	128	> 768	512	> 768

Table (3): Percentage of growth inhibition of MDR pathogenic bacteria by antibiotic combinations

Isolates	Code	Growth inhibition %				
		AK-IMP-FEP	AK-TIG-IMP	TIG-IMP-FEP	AK-TIG-FEP	AK-TIG-IMP-FEP
Proteus sp.	34D	65.7	79.4	68.3	67.9	90.9
Acinetobacter sp.	37D	76.7	86.0	78.5	80.3	91.2
Pseudomonas sp.	38D	79.1	81.6	76.4	83.1	94.4
Staphylococcus sp.	39D	81.9	82.5	89.7	87.6	93.7
Pseudomonas sp.	42D	77.5	80.3	78.9	82.3	95.5
E. coli	46D	86.1	87.2	88.8	88.0	97.6
Pseudomonas sp.	50D	70.8	84.6	77.9	71.7	94.6
Staphylococcus sp.	1F	88.8	90.2	90.7	92.8	98.3
E. coli	16F	74.1	78.8	77.8	82.6	94.6
Klebsiella sp.	35F	79.2	83.6	85.4	86.5	96.3
Staphylococcus sp.	38F	81.8	86.9	85.0	87.9	95.2
E. coli	39F	85.7	87.4	88.3	89.4	98.5

AK = amikacin IMP = imipenem FEP = Cefepime TIG = tigecycline

As the twelve tested isolates in this study were resistance to most of the used antimicrobial agents, single agents did not exhibit any bactericidal activity against tested stains at the used antibiotic concentrations. Moreover, the two antibiotic combinations of the four tested antibiotics at such concentrations against tested isolates achieved < 90% killing. The combination (AK/IMP/TIG/FEP) was the only one which achieved > 90% killing after 24 h exposure against all of the isolates. The three combinations (AK/TIG/IMP, TIG/IMP/FEP and TIG/AK/FEP) also achieved > 90% killing after 24 h exposure against the isolate *Staphylococcus* sp. (1F). The results of the present study were confirmed by the results of other investigators as the following:

Aboulmagd and Alsultan, [23] also found that the tested pathogens were resistance to all the used antimicrobial agents at the used concentrations. Using two antibiotic combinations at the used concentrations against extensively drug resistant (XDR) tested pathogens gave insignificant results. Three combinations (AK/TIG/IMP, TIG/IMP/FEP and TIG/AK/FEP) showed significant bactericidal activity against XDR *A. baumannii* isolates. Only two combinations (IMP/AK/FEP and AK/TIG/IMP) displayed remarkable killing against XDR *P. aeruginosa* isolates. Ugwu *et al*., [12] also found that the combination of the β-lactam antibiotics and gentamicin were synergistic.

Treating these infections with antibiotic combinations is better than using single agent which is confirmed by two recent studies. Mortality rate was lower in patients who received combinations with two or more antimicrobial agent than in those receiving single antibiotic. Another study suggested that using the antibiotic combination of meropenem, tigecycline and colistin reduced mortality rate in patients with bloodstream infections caused by MDR pathogens [25]. Using combinations of antimicrobial agents gave better results than monotherapy which was suggested in one study. Combinations of tigecycline with carbapenems are a preferred option in treating of bacteraemia [26]. The combination of Carbapenem, colistin, and tigecycline have shown good clinical efficacy in the treatment of severe infections caused by XDR pathogens such as invasive infections [27].

4 Conclusion

Investigation of antibacterial activity of antibiotic combinations is necessary because of the lack of novel antimicrobial agents and bacterial resistance to most of commonly used antibiotics. The present study investigated the efficacy of antibiotic combinations on multi-drug resistant pathogens. Our findings suggested that treatment of MDR pathogenic strains with antibiotic combinations maybe an appropriate option.

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