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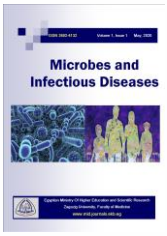

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Original article

Evaluation the combination of amoxyclav with amikacin and ceftriaxone against *Escherichia coli* sepsis

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ABSTRACT

Background: *Escherichia coli* (*E. coli*) is one of the most common causes of bloodstream infections, it is capable of gaining access to and surviving in the bloodstream are known as extra-intestinal. This research was performed to study the effect of some antibiotics on the growth of bacteria by combination with amoxiclav. **Methods:** The bacteria (*E. coli*) was isolated from bloodstream and cultured in three media (blood agar, MacConkey agar, and eosin methylene blue agar), then they were incubated for 24 hours at a temperature of 37 ºC to grow. Well diffusion method is used for antibiotic sensitivity evaluation, after overnight incubation, the diameter of the inhibited growth is measured. Fifty µL of the antibiotics (ceftriaxone, amikacin, and amoxyclav) was added separately in one petri dish, and they were added in another petri dish combined with the amoxyclav, to evaluate the effect of antibiotics on the growth of bacteria before and after the addition of amoxyclav. **Results:** The study showed that males are more infected with 65% of the samples, and it showed that females are less infected, at 35% of the samples without significant differences (p= 0.18). The mean of inhibition zone of amikacin alone was 32.95± 5.95 that increased significantly when combined with amoxyclav 37.40± 5.36 (p= 0.018). Combination of ceftriaxone with amoxyclav showed increased in inhibition zone 43.10± 9.48 than ceftriaxone alone 39.05± 11.55 without significant differences was observed (p= 0.23). Positive strong correlation was observed between amoxyclav and amikacin (r= 0.74, p< 0.001). **Conclusions:** Combination of amikacin with amoxyclav has synergism effect against *E. coli* clinically isolated from patients with bloodstreams (p = 0.018). Addition of ceftriaxone to amoxyclav showed synergism effect but it still insignificant (p = 0.23).

Introduction

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Bloodstream infection (BSI) is defined by positive blood cultures in a patient with systemic signs of infection and may be either secondary to a documented source or primary that is, without identified origin. *Escherichia coli* is considered one of the most important pathogens isolated from bloodstream infections [1].

Escherichia coli is a Gram-negative, non-sporulation, rod-shaped, facultative anaerobic and coliform bacterium pertaining to the genus Escherichia that commonly inhabits the environment, foods, and warm-blooded animals' lower gut [2,3].

In the recent decades overuse and misuse of antibiotics as well as social and economic factors have accelerated the spread of antibiotic-resistant bacteria, making drug treatment ineffective [4]. In the age of increasingly resistant Gram-negative infections, the likelihood that empiric antimicrobial therapy will provide adequate coverage for potential pathogens causing an infection is increased with the use of two antimicrobial agents compared to a single agent [5-7].

Antibiotic susceptibility testing (AST) specifies effective antibiotic dosage and formulates a profile of empirical therapy for the proper management of an individual patient's health against deadly infections [8].

The hydroxyl group creating amoxicillin from ampicillin results in a drug that is more lipid soluble and thus has increased bioavailability and duration of action and, in some pharmacodynamics studies, heightened bactericidal activity [9].

Ceftriaxone is a third-generation cephalosporin antibiotic used for the treatment of a number of bacterial infections. These include middle ear infections, endocarditis, meningitis, pneumonia, bone and joint infections, intra-abdominal infections, skin infections, urinary tract infections, gonorrhea, and pelvic inflammatory disease. Ceftriaxone can be given by injection into a vein or into a muscle [10]. Ceftriaxone showed high resistance rate in the last decades [11].

Aminoglycosides (AGs) have been used for decades as effective agents against most Gram-negative pathogens including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [12-13]. This study is performed to evaluate the antibacterial efficacy of amoxyclav alone and combined with both amikacin and ceftriaxone to treat *E. coli* clinically isolated from patients with blood stream.

Materials and methods

Bacteria were isolated from bloodstream of patients with sepsis. Twenty clinical strains of *E. coli* were acquired from Al- Shomali General Hospital, and were isolated from admitted patients with positive blood cultures. Blood samples were collected before antimicrobial treatment. Blood samples were inoculated immediately under complete aseptic conditions in blood bottles containing 70 mL of brain heart infusion broth, incubated at 37 °C, and examined daily for up to seven days. Subcultures of blood broth were added to blood agar, MacConkey agar, and eosin methylene blue agar (EMB) then incubated aerobically for 24 h at 37 °C. The isolates were identified at first by standard microbiological and biochemical tests [14].

All isolates were identified based on their morphology, Gram staining, biochemical tests, and culture on selective media (EMB agar). Then isolates confirmed by VITEK 2 automated systems (bioMerieux, France).

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts; the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface of the on the muller hinton agar medium. Then, a hole with a diameter of 5 mm is punched aseptically with a sterile cork borer, and a volume (50 µL) of the antimicrobial agent at desired concentration is introduced into the well after they were compared to (Mcfarland standards). Then, agar plates are incubated under suitable conditions depending upon the test microorganism at 37ºC for 18-24 hours. The antimicrobial agent (50 µL) from ceftriaxone 1g/ 5ml, amikacin 500 mg/2ml, and amoxyclav 1.2 g/20 ml diffuse in the well agar medium and inhibits the growth of the tested microbial strain [15].

On January 2, 2023, the study protocol was accepted by the Ethical Committee of the Babylon Health Directorate. Furthermore, the patients' verbal consent was obtained before taking the sample. During the sampling, precautions were taken to ensure the safety of the participants. This work was also carried out by the Iraqi Ministry of Health's Ethics Committee and followed all national rules. The number and date of projects approval were 45239 in January 02, 2022.

For statistical analysis, SPSS software 26 (SPSS Inc., Chicago, USA) was used. Means and standard deviations were used to represent the data. Independent T test was used to examine measurement data with a normal distribution. For correlation analysis, Pearson correlation for parametric analysis was used. *p* value < 0.05 considered as statically significant.

Results

Figure 1 showed the distribution of genders, the study showed that males are more likely to be infected with a rate of 13 samples, or 65% of the total samples, in contrast, it showed that females are less likely to be infected, at a rate of 7 samples, or 35% of the samples without significant differences (*p*= 0.18).

The mean of inhibition zone of amikacin alone was 32.95± 5.95 mm that increased significantly when combined with amoxyclav 37.40± 5.36 mm (*p* = 0.018).

Combination of ceftriaxone with amoxyclav showed increased in inhibition zone 43.10± 9.48 mm than ceftriaxone alone 39.05± 11.55 mm without significant differences was observed (*p*= 0.23) **(Table 1)**.

Positive strong correlation was observed between amoxyclav and amikacin (r= 0.74, *p*< 0.000). As well as strong positive correlation observed between amoxyclav and ceftriaxone (r= 0.78, *p*< 0.001). Amikacin showed strong positive correlation with ceftriaxone (r= 0.58, *p*= 0.007) **(Table 2).**

Figure 1. The distribution of genders among patients.

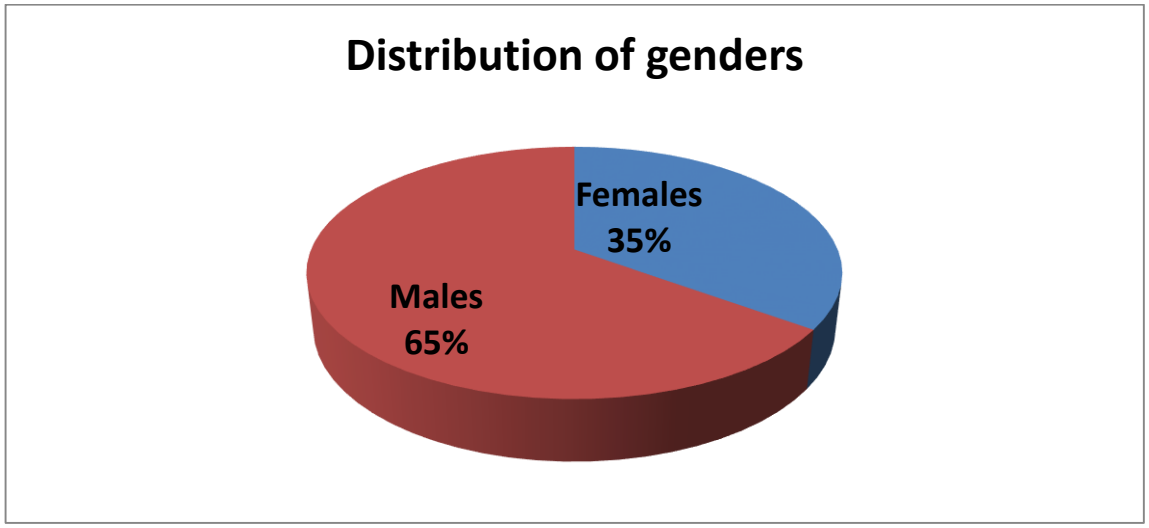

Table 1. Evaluation the effect of amoxyclav combination with other antibiotics.

Groups	N	Mean per millimeters	Std. Deviation	*P* value *
Amikacin only	20	32.95	5.95	**0.018**
Amikacin plus Amoxyclav	20	37.40	5.36	
Ceftriaxone only	20	39.05	11.55	0.23
Ceftriaxone plus Amoxyclav	20	43.10	9.48	

***Independent T test, *p* value < 0.05 considered as statically significant

Table 2. Correlation between different agents.

Variables		Amikacin	Cephalosporin
Amoxyclav	**Correlation**	0.74**	0.78**
	Significance	0.000	0.000
Amikacin	**Correlation**		0.58**
	Significance		0.007

**.Pearson correlation is significant at the 0.01 level (2-tailed).

Discussion

Antibiotics are essential in modern medicine and are used to treat various infectious diseases caused by bacteria. However, the emergence of antibiotic-resistant bacteria has become a global public health threat, leading to increased morbidity, mortality, and healthcare costs. Therefore, it is crucial to determine the antibiotic sensitivity of bacterial strains to choose appropriate antibiotic therapy.

With rapidly increasing antibiotic resistance and decline in new antibiotic drug development, the toughest challenge remains to maintain and preserve the efficacy of currently available antibiotics. Therefore, the best rational approach to fight these infections is to 'hit early and hit hard' and kills drug-susceptible bacteria before they become resistant [16]. The preferred approach is to deploy two antibiotics that produce a stronger effect in combination than if either drug were used alone. Various society guidelines in particular indications also justify and recommend the use of combination of antimicrobial therapy. Combination therapies have distinct advantages over monotherapy in terms of broad coverage, synergistic effect, and prevention of emergence of drug resistance [17].

In the current study, the mean of inhibition zone of amikacin alone was 32.95± 5.95 that increased significantly when combined with amoxyclav 37.40± 5.36 (p= 0.018). Combination of ceftriaxone with amoxyclav showed increased in inhibition zone 43.10± 9.48 than ceftriaxone alone 39.05± 11.55 without significant differences was observed (p= 0.23)**.**

Therapeutic control of P-lactamase-producing bacteria has been a major clinical problem for at least 40 years. Development of drug combinations containing the P-lactamase inhibitors clavulanic acid and sulbactam has given clinicians a crucial approach to controlling resistant organisms on the basis of inhibition studies with isolated enzymes, clavulanic acid is a better inhibitor of the broad-spectrum bacteria. In general, the microbiological spectrum of activity of the two inhibitors combined with an aminopenicillin is similar; however, clavulanic acid is more effective when high enzyme producers are encountered. Because the sulbactam-ampicillin combination is given parenterally, higher blood levels may be attained compared with the orally absorbed clavulanate-amoxicillin formulation. These combinations are well tolerated and represent the opportunity for continued use of the safe and well-established aminopenicillins in infections caused by both aerobic and anaerobic bacteria [18].

Another study mentioned that the four most commonly prescribed antibiotic combinations given to 4451 neonates (77·42%) of 5749 were ampicillin–gentamicin, ceftazidime–amikacin, piperacillin–tazobactam–amikacin, and amoxicillin clavulanate–amikacin. This dataset assessed 476 prescriptions for 442 neonates treated with one of these antibiotic combinations Reported mortality was lower for neonates treated with ceftazidime–amikacin than for neonates treated with ampicillin–gentamicin (hazard ratio [adjusted for clinical variables considered potential confounders to outcomes] 0·32, 95% CI 0·14–0·72; p=0·0060). Susceptibility of Gram-negative isolates to at least one antibiotic in a treatment combination was noted in 111 (28·5%) to ampicillin–gentamicin; 286 (73·3%) to amoxicillin clavulanate–amikacin; 301 (77·2%) to ceftazidime–amikacin; and 312 (80·0%) to piperacillin–tazobactam–amikacin. A probability of target attainment of 80% or more was noted in 26 neonates (33·7% [SD 0·59]) of 78 with ampicillin–gentamicin; 15 (68·0% [3·84]) of 27 with amoxicillin clavulanate–amikacin [19].

In this study, positive strong correlation was observed between amoxyclav and amikacin (r=

0.74, $p< 0.000$). As well as strong positive correlation observed between amoxyclav and ceftriaxone (r= 0.78, $p< 0.001$). Amikacin showed strong positive correlation with ceftriaxone (r= 0.58, $p= 0.007$)

Another study found the 4 antibiotic combinations screened none exhibited synergistic effects on any of the 254 strains. Specifically, the combinations of ampicillin-gentamicin and cefrtiaxone-gentamicin were antagonistic in 1.97% and 1.18% of strains respectively. Similarly, the combinations of ampicillin-tobramycin were antagonistic on 0.78% of all strains. Analysis revealed that an important factor on the responses to the combination treatments was the choice of a specific aminoglycoside over another. Subsequent cross correlation analysis revealed that the interaction profiles of combinations including the same aminoglycoside are significantly correlated ($p<0.001$) [20].

Conclusions

Combination of amikacin with amoxyclav has synergism effect against *E. coli* clinically isolated from patients with blood streams ($p= 0.018$). Addition of ceftriaxone to amoxyclav showed synergism effect but it still insignificant ($p= 0.23$).

Limitation of the study

The main limitation in this study is the absence of genotyping study of the bacteria for participants; the current study took in consideration only phenotypic properties. Small numbers of specimens may not reflect all population in Babylon city.

Conflict of interest

None declared.

Funding

None declared.

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