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Effect of β lactam Antibiotics with Aminoglycosides on Multidrug Resistance *Staphylococcus aureus*

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Abstract : A total of two hundred and ninety samples were collected from midstream urine taken from patients suffering from urinary tract infections**,**21isolates of *Staphylococcus aureus* were isolated and identified depending on their morphological properties (cultural and microscopical) and biochemical tests. The antibiotic sensitivity of the isolates is tested against twenty antibiotics, the isolates showed high resistance. The meropenem, impinem amikacin, nitrofurantion, cifotaxim, and sifitrixone are found more effective. The inhibition concentrations of isolates were higher. The combination of *β*lactam with an aminoglycoside (Amikacin and gentamycin) antibiotics showed an active synergistic effect against the multidrugs resistance of isolate.
Keywords: Minimum Inhibitory Concentration and Combination, Antibiotics. *S. aureus*.

Introduction

Urinary tract infections are a community-acquired bacterial disease which frequently affects human especially female outpatients. *S. Aureus* is the most common member of the family staphylococcus accounts for the majority of all UTI in both inpatients and outpatients[1]. *Staphylococcus spp*. are a normal flora of human skin and mucous membranes and their spread, is either endogenously or from infected skin, It is an opportunistic organism that is able to cause the infections, mainly in compromised hosts, it has many species but the main three species are *S. aureus, S. epidermidis* and *S. saprophyticus*. The pathogenicity of *S. aureus* is contributed to hemolysis of the blood, coagulation of the plasma, production of extracellular toxin, enzyme, and formation biofilm on medical device which consider one of its virulence factors, has become the common problem, because of the development in the resistance mechanisms of antibiotics[2]. *S. aureus* presented significantly in a greater percentage of people in the hospital setting that the carrier state serves as a reservoir for infection of hospitalized patients[3] .Clinically significant methicillin-resistant *S. aureus* (MRSA) causes many infections such as nosocomial infections and hospital–acquired UTI, these bacteria is innate resistance to antibiotics and disinfectants, antibiotic resistance with microorganism has become the worldwide concern.It is either natural or acquired, transmissible antibiotic resistance is the major cause of concern as it can lead to rapid spread of antibiotic resistance and has proven difficult to eradicate[4].Accordingto clinical importance for *S. aureus* represented by its ability on causes disease and multidrug resistance. Present study aimed to find difference cause effective on multidrug resistant *S. aureus,* represented by a combination of *β*–lactam with aminoglycosides antibiotics.

Materials and Methods

209 clinical samples are collected from Hilla Teaching Hospital, Babylon Maternity and Pediatrics Hospital, Hilla, Iraq of both sexes and various ages. These samples were placed in sterile containers, individually and then transferred to the laboratory for the next experiments.*S. aureus* isolates were isolated on blood agar *base* and MacConkey agar using streaking techniqueand identified depending on their morphological properties such as cultural and microscopical and biochemical tests[5].Twenty antibiotics (Bioanalyse-Turky) were used in this test according to [6].The concentrations of these antibiotics were as follows: 5 µg/disk for rifampicin, methicillin, norfloxacin and ciprofloxacin, 10 µg/disk for penicillin G, meropenem, imipenem, lomefloxacin, tetracycline, gentamycin, for erythromycin, 25 µg/disk for amoxicillin, 30 µg/disk vancomycin, chloramphenicol, ceftriexone, cefotaxime, ceftazidime, amikacin,50 µg/disk for trimethoprim. Blood agar base (Oxoid) is used for detection the ability of the isolates to produce the haemolysin[5].Urea agar base (Himedia) is used for detection the ability of isolates to produce the urea[5].The (MIC) of some antibiotics were tested against the bacterial isolates depending on the washed turbidity of the bacterial growth by naked eye. The MIC is recorded as the lowest concentrations prevent bacteria under test to grow, according to the method recommended [7].This test worked according to Half–Chess board technique, suitable concentrations of antibiotics in this test ofcidal activity(10µg/ml)for amoxicillin, cifotaxim, siftrixon,(5µg/ml) for amikacin and gentamycin recommended [8].

Results and Discussion

A total of Twenty one isolates of *S. aureus* were isolated from 290 clinical samples, which isolated from urine. The details of distribution and percentages of the isolates on the samples were summarized in Table 1.Dharmadhikari and Peshwe, 2009refersto isolate two isolates of *S. aureus* from the urine of UTI patients and it is multiresistant to different antibiotics[9]. Mansour et al. 2009isolated 25 isolates(24.5%) of *S. aureus* from553(87%) patients out of 7056 were shown to be urine culture positive from UTI patients[10].

Table 1.The distribution and percentages of *S. aureus* isolate

Bacterial Isolates Isolated from Midstream urine	**No.Isolates**				**Total**	**%**
	Male	**Male Childern**	**Female**	**Female Childern**		
S. aureus	7	0	11	3	21	7.2
No. Samples	58	9	92	48	207	71.4
Positive Culture	67		140			
Negative Culture	25	5	45	8	83	28.6
Total	97		193		290	100

Table 2.The diagnostic and biochemical tests of *S. aureus*

Test	***S. aureus***
Gram stain	G+vecocci (clusters)
Catalase	+
Oxides	–
Urease	–
Nitrate	+
VogesProskauer's	+
Arginine utilization	+
Glucose Fermentation	+
Trehalose fermentation	+
Maltose fermentation	+
Lactose Fermentation	+
Raffinose fermentation	-
Haemolysis RBC	*β*
Production plasma coagulation enzyme	+
Production pigment	Yellow
Mannitol ferment and growth in salt concentration	+
Sensitive to Novobiocin	S
Motility	–

Positive Result: +,negative Result,- :Complete Himalayas:***β***

S. aureus consider of the most important strains recovered from a UTI, showed the highest degree of resistance to most antibiotics, especially *B*-lactam antibiotics and causing severe and relatively more serious infections especially which related to hospitals. The main reasons for the resistance of the *B*-lactam antibiotics may be attributed to the degradation of these antibiotics by *B*-lactamase enzymes, which is normally plasmid encoded, lack of penicillin binding protein for specific antibiotics or due to the change of drug permeability. Twenty-one isolate of *S.aureus* are tested against twenty common antibiotics as shown in Tables 2 and 3. It is found that most isolates were resistant to the antibiotics, especially to*B*-lactam antibiotics. The *S.aure*us isolate No.2 was resistant to 16 antibiotics. Whereas, the isolate No. 17 was sensitive to most antibiotics. The isolates showed resistant to penicillin G, amoxicillin, and methicillin. It is resistant to methicillin defined as methicillin resistant *S. aureus* (MRSA).Itsresistance to penicillin is mediated by penicillin's production: an enzyme, which broke down the β-lactam ring of the penicillin. It cannot bind to penicillin binding proteins (PBPs)on bacterial cell wall [2].

Table 3.The antibiotic susceptibility of *S.aureus* isolated from UTI

Antibiotic / No. Isolate	Meropenem	Impenem	Vancomycin	Sefitryxon	Rifampicin	Norfloxacin	Trimethprim	Lomfloxacin	Amoxicillin	Erethromycin	Gentamycin	Cloramphenic	Ceftazidime	Ciprofloxacin	Tetracycline	Penicillin G	Amikacin	methicillin	Nitrofurantion	Cefotaxim
1	-	-	+	I	+	-	+	+	+	+	+	-	+	-	+	+	-	+	+	+
2	-	I	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+
3	-	-	-	-	+	-	+	-	+	+	-	+	-	+	+	+	-	+	+	-
4	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	+	-	+	-	+
5	-	-	-	-	-	+	-	-	+	-	-	+	+	+	+	+	-	+	-	-
6	-	-	+	-	-	+	+	-	+	I	-	+	-	-	+	+	-	+	-	-
7	-	-	+	-	-	+	-	-	+	+	-	-	-	-	-	+	+	+	-	-
8	-	-	-	+	-	-	+	I	+	+	-	-	-	-	+	+	I	+	-	-
9	-	-	+	-	-	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+
10	-	-	+	-	-	+	+	-	+	+	-	-	-	I	+	+	-	+	-	-
11	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	+	-	+	+	-
12	-	-	-	-	-	+	+	-	+	+	-	+	-	-	+	+	-	+	-	-
13	-	-	+	-	-	+	-	+	+	-	-	+	+	+	+	+	-	+	-	-
14	-	-	-	-	-	-	-	I	+	+	-	-	-	-	-	+	I	+	+	-
15	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
16	-	-	-	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	-	-
17	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	+	-	+	-	-
18	-	-	-	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	-	+
19	-	-	+	-	-	-	+	+	+	+	+	+	-	+	+	+	-	+	+	-
20	-	-	+	-	-	+	+	+	+	I	+	+	+	-	+	+	+	+	-	-
21	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-

Abbreviations: + resistance, - sensitive, I: moderate resistance.

Mansour et al. 2009reported that (25%) of *S. aureus* is resist to sifitrixone and ciprofloxacin, (75%), (50%), and (69.2%) are resist to cifotaxime, gentamycin and amikacin respectively[10]. Whereas, the isolates in present study showed resist to trimethprim, tetracycline and erythromycin(76.2%), chloramphenicol (71.4%)Co-trimoxazol(61.9%).

The current study revealed that imipenem, meropenem, amikacin, gentamycin, rifampicin, cefotaxim, sefitrixon, ciprofloxacin, lomfloxacin, and nitrofurantion were the most effected antibiotics on MRSA isolates. Resistance of *S.aureus* to quinolones is a chromosomally mediated, and occurs by mutation of the basic genetic material that code for the gyrases, another mechanism of resistance is impaired penetration of the outer membrane of the organism. It have efflux pump,Multi drug resistant bacteria such as *S. aureus* have inherent nature of developing resistant strains for most antibiotics.Italso have thick cell wall contains peptidoglycan teichoic acid and lipoteichoic acid, capsular and alginate materials which facilitated an adherence of bacteria to epithelium of urogenital tract ,staphylococci spp. involved in the pathogenesis of chronic pelvic pain syndrome [2]. *S.aureus* exhibited resistance to most antibiotics such as aminoglycosides, pincillines, quinolones, chloramphenicol, chloramphenicole, disinfectants, and heavy metals may be attributed to the R-plasmid, transposons and integrons acquired from pathogenic bacteria present in the site of infection as multi drug resistant *P.aeruginosa*, this isolates in this study may acts as a reservoir for resistance, which it transferred to *Staphalococcal spp*. and others microorganisms. The transfer of resistance among different genera and species of Gram-positive and Gram-negative bacteria[11]. Transmissible antibiotics resistance is the main cause of concern as it lead to rapid spread of antibiotics resistance and has proven difficult to eradicate. *S.aureus* isolates showed(19.0%),(23.8%) resistant to Sefitrixone and cefotaxim and (14.2%), (33.3%)to amikacin and gentamycin, respectively. *S.aureus* isolates were resisting to norfloxacin and sefitrixone 25% and 17%,

respectively[9]. All cephalosporins and penicillins require binding to a penicillin-binding protein located in the bacterial cell wall to initiate their activity.(MRSA) produced a defective low-affinity PBP2a due to the presence and activation of the *mecA* gene, which was borne on plasmid DNA**.**Cephalosporins and penicillins binding affinity to PBP2a is very low disabling. It can to disrupt cell wall synthesis and rendering the drug ineffective, other mechanisms were regarded to cell wall thickening [12].*S.aureus* isolates showed(47.6%)resistant to vancomycin, and called vancomycin resistant *S.aureus*(VRSA).Mechanism for vancomycin resistance by *S.aureus* through alteration in the molecular structure to cells wall precursorcomponents, which decreases binding of vancomycin so that allowing the cell wall synthesis to be continued[13].In the present study, twelve (10) antibiotics were tested for Minimum Inhibitory Concentrations (MICs). These antibiotics are trimethoprim, rifampin, ciprofloxacin, penicillin G, Tetracycline cefotaxime, gentamicin, ceftriaxone, methicillin, norfloxacin. These antibiotics were chosen as they are traditionally used in the treatment of bacterial infections especially UTI. In the dilution method, various concentrations of antibiotics are inoculated with a standard suspension of tested MRSA isolates. Following an overnight incubation at 37°C,the(MIC) is detected by observing the lowest concentration of the antibiotic that will inhibit visible growth of the tested bacteria[14].The results showed a high degree of Staphylococcal resistance to most antibiotics used in this study as shown in Table 4.

Table 4. MICs of some antibiotics against MRSA Isolates

Antibiotics	% for resistant bacteria	MICs values µg/ml
Tp	16(76.2)	16->128
Rif	5(23.8)	4-128
Tf	(38.3)7	0.5-64
P	(100)21	64->128
Te	(76.2)16	32->128
Ctx	(23.8)4	8-128
Gn	(33.3)7	0.25-<128
Sxt	(19.0)4	0.5-64
Me	(100)21	64->128
Nor	(57.1)12	1->128

MICs ranged from16 to >128, 32 to >128µg/ml for trimetheprim, tetracycline respectively, and64 to >128 µg/ml for penicillin G and methicillin and1 to >128.4 -128 and to 8-128µg/ml for norfloxacin, rifampicin and cefotaxim respectively, while MRSA showed resistance for sefitrixone and ntrofurantion0.5-64 µg/ml. The purpose of use antibiotics combinations to treat severe bacterial infections, especially multidrug resistance bacteria, reduce the chance of resistant mutants arising during the treatment by monotherapy and may be more effective than other drugs when used alone. A combination between any two antibiotics may be either synergistic, partially synergistic (additive), indifferent or antagonistic[7]. Antibiotics combinations can achieve synergistic or additive effects are of great value when toxicity of one or both members of the combination is considered because with the enhanced effect one can use low concentrations to produce the desired therapeutic action, Many important antibiotics have significant dose-related toxicities which seriously limit their use(e.g. ciphalosporines, aminoglycosides and chloramphenicol)[15, 16]. The antibiotics combination for two selectedisolates of MRSA was investigated and the results refers to synergistic effects between beta-lactam antibiotics represented by amoxicillin, cephalosporines antibiotics represented by cefotaxime and sulfonamide derivative represented by ceftriaxone with aminoglycosides Tables 5 and 6. the beta-lactam antibiotics are the safest and mostly widely used in treatment UTI, known to bind with specific receptors in the cell wall which called penicillin binding proteins, after antibiotic molecules attached to the receptors, peptidoglycan synthesis is inhibited and transpeptidation is blocked and activates the autolytic enzymes and results in cell lysis. The results showed that patients with severe multidrug resistant *S. aureus* UTI infections should be treated with combination antibiotics, consisting of an antistaphylococcal beta-lactam, cephalosporines and sulfonamide derivative with an aminoglycoside to provide adequate therapy and improve patient outcomes[15, 16].

Table 6.Antibiotics Combination Effect on UTI isolate No.2 of MRSA

Antibiotics	**Amc**	**Ctx**	**Sxt**	**Ak**	**Gn**
Amx	++	++	-	-	+
Ctx		++	(+)	-	+
Sxt			-	-	-
AK				-	(+)
Gn					++

++:Static effect only + :Partial cidal effect
(+):Incomplete cidal effect - :Fully cidal effect

Table 7.Antibiotics Combination Effect on UTI isolates No.9 of MRSA

Antibiotics	**Amc**	**Ctx**	**Sxt**	**Ak**	**Gn**
Amx	+ +	(+)	-	-	(+)
Ctx			+	-	-
Sxt			(+)	-	-
Ak				(+)	(+)
Gn					++

++:Static effect only +:Partial cidal effect
(+):Incomplete cidal effect -:Fully cidal effect

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