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# The role of combination of Meropenem with gentamicin in reducing emergence of antibiotic resistance in some highly resistant staphylococcus species *in vitro*

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**Abstract:**

Background: Staphylococci are considered clinically one of the most important pathogens causes pneumonia, meningitis, boils, arthritis and osteomyelitis. As well as keratitis and urinary tract infections. Emergence of resistant strains of Staphylococcus for a wide range of antibiotics considered a major dilemma leading to failure of infections treatment. The famous example is Methicillin Resistant *Staph aureus* (MRSA). Meropenem is one of the carbapenem antimicrobials acts through inhibiting bacterial cell wall synthesis. Gentamicin is one of aminoglycosides group act through inhibiting of direct primarily bacterial protein synthesis. **Aim** of this study is to evaluate the effect of meropenem alone and in combination with gentamicin on *Staphylococc*i to determine the efficacy of combination of meropenem and gentamicin on prevention the emergence of resistant *Staphylococcus*. Materials and methods: fifty Samples collected by culturing urine samples and scraping infected cornea of patients attained to Al-Kadhimia and Ibn-Al Haitham eye hospitals for period between April to September/2011,identification of staphylococcus depend on morphological tests using gram's stain smears and colonies morphology , biochemical tests including and API staph system. The disc diffusion method was used to test the antibiotic sensitivity of the isolates. Broth dilution method was used to determine the minimum inhibitory concentration (MIC) of Meropenem, Gentamicin, and their combinations. Fractional inhibitory concentration (FIC) values were used to assess the synergism between meropenem and gentamicin. Two isolates (*Staph. aureus* and *Staph. epidermidis*) was cultivate in 1/4 MIC of meropenem and gentamicin for 18 hours and repeating this step for consequent seven times. After the 7th passage the new MIC value of Meropenem and gentamicin for these two isolates were measured. Results: *Staphylococcus* species was 13 of all isolates (26 %). Percentage of resistance for the tested antibiotics were Bacitracin 15 % ; : Trimethoprim 30.7 ; Kanamycin 61% ; Vancomycin 30.7 ; Cephalexine 46% ; Chloromphenicol 23 % ; Streptomycin 23 % ; Rifampicin 7.7 % ; Ciprofloxacin 23 % ; Gentamicin 46 % ; Meropenem 15.3 %; Oxacillin 7.7 %. MIC values for meropenem were 0.5- 4 µg/ml for sensitive isolates (84.7 %) and 9-12 µg/ml for resistant isolates (15.3 %), while for gentamicin 2-4 µg/ml for sensitive isolates (54 %) and 24-50 µg/ml for resistant isolates ( 46 % ). FIC values for three isolates were 2 ( no effect for combination) while the values were 0.5 (synergistic effect ) for 11 isolates. Passing *Staphylococci* in 1/4 MIC for seven days increase the MIC values for meropenem from 2 µg/ml to >250 µg/ml in *Staph aureus* and from 4 µg/ml to >250 µg/ml in *Staph epidermidis*, and for gentamicin from 6 µg/ml to >250 µg/ml in *Staph aureus* and from 4 µg/ml to >250

µg/ml in *Staph epidermidis*. The results of this study revealed the synergistic activity of combinations of meropenem and gentamicin on *Staph aureus* and *Staph epidermidis*, even with sub-inhibitory concentration (1/4 + 1/4; 1/2 + 1/2). Such combinations will reduce prevalence of resistance and improve therapeutic outcomes and may prevent the development of resistance.

## دور مزيج الميروبينيم و الجنتامايسين في تقليل نشوء مقاومة المضادات الحيوية في بعض أنواع المكورات العنقودية عالية المقاومة ، دراسة في الزجاج

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**الخلاصة:**

خلفية الدراسة : تعد المكورات العنقودية واحدة من أهم العوامل المرضية سريريا" و التي قد تسبب ذات الرئة و ألتهاب الاغشية الدماغية و الدمامل و ألتهاب المفاصل و ألتهاب نقي العظم بالاضافة الى ألتهاب القرنية و خمج القنوات البولية.أن نشوء عتر مقاومة من المكورات العنقودية لكثير من المضادات الحيوية يعد معضلة رئيسية تؤدي الى فشل علاج العدوى بالجراثيم ، و المثال الاشهر لذلك هو المكورات العنقودية الذهبية المقاومة للمثيسيلين ( MRSA) . أن الميرومبينيم من مجموعة الكاربابينيم و يعمل بتثبيط تخليق جدار الخلية الجرثومية ، والجنتامايسين من مجموعة الامينوكلايكوسيدات و يعمل بتثبيط تخليق بروتين الخلية البكتيرية. **هدفت** هذه الدراسة الى تقييم تأثير الميروبينيم لوحده وعند مزجه مع الجنتامايسين على عزلات المكورات العنقودية لتحديد فعالية المزيج في منع نشوء المقاومة أو تقليل ذلك. المواد وطرق العمل: جمعت 50 عزلة جرثومية بزرع عينات أدرار و كشطات من القرنيات المخمجة من المرضى الذين راجعوا مستشفى الكاظمية و مستشفى أبن الهيثم لطب العيون للمدة من نيسان الى أيلول / 2011 . أعتمد تشخيص المكورات العنقودية على الفحوص الشكلية و صبغة كرام و شكل المستعمرات الجرثومية و كذلك الفحوص الكيموحيوية المتضمنة API Staph System . أستخدمت أقراص المضادات الجرثومية لاختبارالعزلات الجرثومية للمضادات الحيوية ، و لتحديد التركيز المثبط الادنى (MIC ) للميروبينيم و الجنتامايسين و مزيجاتهما أستخدمت طريقة تخفيف المرق المغذي ، بينما أعتمد منسب التثبيط الجرثومي ( FIC ) لتقدير التآزر في مزيجات المضادين. و لأختبار تأثير تمرير الجراثيم في تراكيز دون MIC زرع نوعين من المكورات العنقودية *Staph aureus* و *Staph epidermidis* في 1/4 التركيز المثبط الادنى لكل من المضادين موضوع الدراسة و تكرر الزرع لسبع مرات متواصلة و لمدة 18 ساعة لكل منها و بعد التمرير السابع تم قياس قيم MIC الجديدة لكلا المضادين. النتائج: كانت نسبة المكورات العنقودية 26% (13 عزلة) من العزلات الخمسين. و وجد ان نسبة المقاومة للمضادات المفحوصة كالآتي:الباسيتيرسين 15% والترايميثوبريم 30.7% والكانامايسين 61% والفانكومايسين 30.7% والسيفالكسين 46% والكلورمفنيكول 23% والستروبتومايسين 23% والريفامبسين 7.7% والسبروفلوكساسين 23% والجنتاماسين 46% والميروبينيم 15.3% والأوكساسيلين 7.7% . وكان التركيز المثبط الادنى للميروبينيم 0.5 - 4 مايكروغرام/مل وبنسبة (84.7%) للعزلات الحساسة وفي العزلات المقاومة وجد 9- 12 مايكروغرام/مل بنسبة (15.3%) . أما الجنتامايسين فكان التركيز المثبط الأدنى له للعتر الحساسة 2 – 4 مايكروغرام / مل بنسبة (54%) و 24- 50 مايكروغرام /مل للعتر المقاومة (46%) . وكانت قيم منسب التثبيط الجرثومي (FIC) لثلاث عزلات عالية المقاومة 2 اذ لم يكن لمزج المضادات أي تأثير تآزري فيها بينما بلغت قيمة FIC 0.5 لإحدى عشرة عزلة (تأثير تآزري). نتج من تمرير جراثيم المكورات في 1/4 التركيز المثبط الادنى لسبعة مرات متتالية زيادة في قيمة MIC للميروبينيم من 2مايكروغرام \ مل الى > 250 مايكروغرام / مل و من 4 مايكروغرام \ مل الى > 250 مايكروغرام \ مل لكل من ال *Staph aureus* وال *Staph epidermidis* على التوالي وفي حالة الجنتمايسين ارتفعMIC من 6 مايكروغرام \ مل الى > 250 ومن 4 مايكروغرام \ مل الى > 250 مايكروغرام \ مل لكل من ال *Staph aureus* وال *Staph epidermidis* على التوالي . أظهرت نتائج هذه الدراسة تأثيرا" تآزريا" لمزيج الميروبينيم والجنتمايسين على المكورات العنقودية وبتراكيز أقل من التركيز المثبط الادنى 1/4 , 1/2 +1/2 (MIC +1/4) ومثل هذا المزيج سيقلل من نسبة المقاومة ويحسن الناتج العلاجي وربما سيمنع تطور المقاومة الجرثومية لهذه المضادات.

### Introduction:

Staphylococci are considered clinically one of the most important genera of micrococacea family where its pathogenic effect mainly associated with the toxins

that it produces. It has been found to be the causative agent of pneumonia, meningitis, boils, arthritis and osteomyelitis. (1) Emergence of resistant strains of Staphylococcus for a wide range of antibiotics considered a major dilemma leading to failure of infections treatment (2). The famous example is Methicillin Resistant *Staph aureus* (MRSA) (2). Meropenem is one of the carbapenem antimicrobials act through inhibiting bacterial cell wall synthesis (3) . Gentamicin is one of aminoglycosides group act through inhibiting of direct primarily bacterial protein synthesis (4). Aim of this study is to evaluate Meropenem alone and in combination with Gentamicin on *Staphylococc*i to determine the efficacy of combination of Meropenem and Gentamicin on prevention of emergence of resistance *Staphylococcus aureus* and *Staphylococcus epidermis* isolated from cases of keratitis and other UTI of patients attainted to AL-Kadhimia and Ibn Al-Haitham eye hospital in Baghdad city.

**Materials and methods:**

Fifty Samples collected by culturing urine samples and scraping infected cornea of patients attained to Al-Kadhimia and Ibn-Al Haitham eye hospitals for period between April to September / 2011. Identification of staphylococcus depend on morphological tests using gram's stain smears and colonies morphology on brain heart infusion agar (5) , biochemical tests including (catalase test , coagulase test , growth on mannitol salt agar) (6) and API staph system ( Biomeriux company 2003). (7).

**-Susceptibility testing:**

The disc diffusion method was used to test the antibiotic sensitivity of the isolates(6) using the following antibiotic discs {Bioanalyse - turkey} (Kanamycin, Cephalexine, Vancomycin, Trimetheprim, Chloramphenicol, Streptomycin, Gentamicin, Ciprofloxacin, Bacitracin, Imipenem, Rifampicin and Oxacillin) where bacterial solution adjusted with McFarland solution number 0.5 and Muller-Hinton agar{oxoid- England } used as a media.

Agar dilution method was used to determine the minimum inhibitory concentration (MIC) of (Meropenem, Gentamicin, and the combinations of meropenem with Gentamicin in different ratios) by using McFarland solution number 0.5 as a standard inoculums and brain heart infusion agar as a bacterial media(8).

**-Fractional inhibitory concentration (FIC) values:**

FIC values was used to assess the synergism between Meropenem and Gentamicin which calculated from determination the MIC for each agent before and after combinations using the following equation: FIC = (MIC of Meropenem in combination/ MIC of Meropenem alone )+ (MIC of Gentamicin in combination / MIC of Gentamicin alone) synergism was defined as an FIC index of ≤0.5, indifference was defined as FIC index > 0.5 to 4, antagonism was defined as an FIC index >4 (9) .

**-Determination the significance of development of staphylococcal spp. resistance to the Meropenem:**

Two isolates selected, one was *Staphylococcus aureus* and the other was *Staphylococcus epidermis* and the MIC value of meropenem and gentamicin for each isolate determined. Then each isolate was cultivate in quarter MIC of meropenem and gentamicin for 18 hours and repeating this step for consequent seven times. After the 7th passage the new MIC values of Meropenem and gentamicin for these two isolates were measured.

**Results:**

Fifty isolates collected form infected keratitis in Ibn Al Haitham eye hospital and Al-Kadhimia hospital in Baghdad form both genders for period between April to

September/2011, staphylococcus species was 13 of all isolates.

**Table (1):** Types of Staphylococcus isolates.

| Isolate No. | Type of staphylococcus | Isolate No. | Type of staphylococcus |
|---|---|---|---|
| **1** | Staph. epidermids | **8** | Staph. aureus |
| **2** | Staph. epidermids | **9** | Staph. epidermids |
| **3** | Staph. epidermids | **10** | Staph. aureus |
| **4** | Staph. aureus | **11** | Staph. epidermids |
| **5** | Staph. epidermids | **12** | Staph. epidermids |
| **6** | Staph. epidermids | **13** | Staph. epidermids |
| **7** | Staph. epidermids | | |

The percentage of resistance of Staphylococcus isolates to different antibiotics were assisted by antibiotic disc diffusion method ( table 2 ).

**Table (2) :** Percentage of resistance of staphylococcus isolates to different antibiotics.

| Isolates No. | Types of antibiotics | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | TMB | K | VA | CL | C | S | RA | CIP | CN | MPM | OX |
| **1** | R | R | R | R | R | S | S | R | R | R | R | S |
| **2** | I | S | R | I | R | S | I | S | R | R | R | S |
| **3** | I | R | R | S | R | I | S | S | R | R | S | S |
| **4** | I | S | R | I | R | R | I | S | S | I | S | S |
| **5** | R | S | R | S | S | S | I | S | I | S | S | S |
| **6** | I | S | S | R | S | S | I | S | S | R | S | S |
| **7** | I | S | S | R | S | R | S | S | I | S | S | S |
| **8** | S | S | S | R | S | S | S | S | I | R | S | S |
| **9** | I | R | R | I | R | I | R | S | S | S | S | S |
| **10** | I | S | R | S | I | I | R | S | S | I | S | R |
| **11** | S | S | I | S | I | S | I | S | S | S | S | S |
| **12** | I | R | S | S | R | S | S | S | I | R | S | S |
| **13** | I | S | R | I | I | R | R | S | S | S | S | S |
| **% of resistance** | 15 % | 30.7 % | 61 % | 30.7 % | 46 % | 23 % | 23 % | 7.7 % | 23 % | 46 % | 15.3 % | 7.7 % |

Whereas R= resistant, I= intermediate, S= sensitive according to NCCLS 1993
B: Bacitracin ; TMB: Trimethoprim ; K: Kanamycin ; VA: Vancomycin ; CL: Cephalexine
C : Chloromphenicol ; S : Streptomycin ; RA: Rifampicin ; CIP: Ciprofloxacin ;
CN : Gentamicin ; MPM : Meropenem ; OX : Oxacillin

MIC values of Meropenem and Gentamicin for Staph. isolates were measured (table 3).

**Table (3) :** MICs of meropenem and Gentamicin for Staph. Isolates.

| Isolates No. | MIC µ/ml | |
|---|---|---|
| | **Meropenem** | **Gentamicin** |
| **1** | 12 (R) | 50 (R) |
| **2** | 9 (R) | 50 (R) |
| **3** | 1 (S) | 50 (R) |
| **4** | 2 (S) | 6 (I) |
| **5** | 4 (S) | 4 (S) |
| **6** | 0.5 (S) | 38 (R) |
| **7** | 2 (S) | 4 (S) |
| **8** | 4 (S) | 24 (R) |
| **9** | 2 (S) | 2 (S) |
| **10** | 1.0 (S) | 6 (I) |
| **11** | 4 (S) | 2 (S) |
| **12** | 4 (S) | 30 (R) |
| **13** | 4(S) | 4 (S) |
| **%of resistance** | 15.3 % | 46% |

S= sensitive, R= resist, I= intermediate according to MIC break points where [gentamicin S<4, R>8; Meropenem S<4, R>8] (NCCLS, 2003).

Fractional inhibitory concentrations (FIC) of combination of Meropenem with gentamicin was assisted by culturing Staph. isolates in three combinations (table 4).

**Table (4) :** Fractional inhibitory concentration (FIC) of combinations of meropenem and gentamicin.

| Isolates No. | Effect of different combinations of Meropenem with gentamicin | | | FIC values |
|---|---|---|---|---|
| | **½+½ MIC** | **¼+¼ MIC** | **⅛+⅛ MIC** | |
| **1** | + | + | + | 2 |
| **2** | + | + | + | 2 |
| **3** | + | + | + | 2 |
| **4** | - | - | + | 0.5 |
| **5** | - | - | + | 0.5 |
| **6** | - | - | + | 0.5 |
| **7** | - | - | + | 0.5 |
| **8** | - | - | + | 0.5 |
| **9** | - | - | + | 0.5 |
| **10** | - | - | + | 0.5 |
| **11** | - | - | + | 0.5 |
| **12** | - | - | + | 0.5 |
| **13** | - | - | + | 0.5 |

MIC values of meropenem and gentamicin for isolates of staphylococcus before and after exposure to quarter MIC of meropenem for 7 days were measured.

**Table (5):** Effect of passing staphylococci in 1/4 MIC for seven days.

| Staphylococcal Isolates | Initial MIC values in (µg/ml) | | MIC value in (µg/ml) after the 7th day of culturing in 1/4 MIC | |
|---|---|---|---|---|
| | Meropenem | gentamicin | Meropenem | Gentamicin |
| Staph aureus | 2 | 6 | >250 | >250 |
| Staph epidermis | 4 | 4 | >250 | >250 |

**Discussion:**

The results of this study revealed that staphylococcus resemble a considerable percentage of causative agents of keratitis and UTI (26 %) (table 1). This relatively high percentage may attributed to the presence of Staphylococcus as a part of body normal flora which can cause opportunistic infection (10) , and it has many surface antigens, toxins, enzymes, which facilitate its invasion of body tissue and causing infection(11) .

The antibiotic disc sensitivity results (table 2 ) show that most isolates were found resistant to multiple antibiotics which had different mechanisms of action and these results were in agreement with that found by other researchers. (12,13) The high percentage of the antibiotic resistance probably be due to different mechanisms, like enzymes inactivation of antibiotics, alteration of target receptor sites, reduction of influx and increasing of efflux mechanisms. (12) Although many research work illustrated the higher activity of meropenem (and carbapenem group) against different types of bacteria (14), a considerable percentage resistant staphylococci isolates for meropenem were found in this study. This resistance could be attributed to three mechanisms : reduced permeability, efflux, and synthesis of carbapenem β-lactamases (15) . The results of this study were in agreement with other researches (16) in which they found that the percentage of resistant staphylococci to gentamicin was 45 %, That could be attributed to the extensive and frequent use of this antibiotic in inappropriate way, where antibiotic resistance is a function of drug exposure and can be acquired through numerous mechanisms. (17)

Minimum Inhibitory concentration (MIC) of each meropenem and gentamicin for each isolate were measured (table 3), FIC values were determined for combinations of ( 1/2 + 1/2 ; 1/4 + 1/4 ; 1/8 + 1/8 MIC ) ( table 4). The two antibiotics showing 77 % of synergism in combinations ( 1/2 +1/2 ; 1/4 +1/4 ), while no effect was noticed for ( 1/8 + 1/8 ) combinations. This synergism may be attributed to the mode of action of meropenem on the cell wall of bacteria that facilitate the entrance of gentamicin into the bacterial cell and overcome the resistance mechanism. These results were in agreement with other research results. (18)

Culturing the bacterial isolates in media containing sub-inhibitory concentration of certain antibiotic (1/4 MIC for several passages) provoked multiplying bacterial cell to develop the resistance mechanisms against that antibiotic (table 5), this may be a simulation of the inappropriate administration of antibiotics and the abuse of antibiotic use, especially in Iraq since

anyone could buy antibiotics without prescription and those which prescribed are mostly without antibiotic sensitivity test . The results of this study revealed the synergistic activity of combinations of meropenem and gentamicin, even with sub-inhibitory concentration ( 1/4 + 1/4 ; 1/2 + 1/2 ). This is important, since the activity of these antibiotics were improved against sensitive, resistant, and multidrug resistant staphylococcal isolates. Such combinations will reduce prevalence of resistance and improve therapeutic outcomes and may prevent the development of resistance. (19)

**References:**

1- Ryan K. J. and Ray C. G. .. *Sherris Medical Microbiology* (2004): 4th ed. McGraw Hill.

2- Brumfitt W and Hamilton-Miller J.. *Methicillin-resistant Staphylococcus aureus* : N Engl J Med (1989)320:1188–1196.

3- Henry F and Chambers, MD: Basic and clinical pharmacology . 10th ed. In : Bertram G. and Katzung M.D. (2007) Lang medical book/ Mc Graw Hill. PP. 726-770.

4- Falagas, ME; Grammatikos, AP.; and Michalopoulos, A.: Potential of old generation antibiotics to address current need for new antibiotics. (2008) Expert. Rev. Anti. Infect. Ther.
6 (5): 593-600.

5- Koneman E. W. ,Allen S A , Janada W M , Schreckemberger P C and Winn J R .. *Color plate and textbook of diagnostic Microbiology.* (1992) *4th ed.* . Washington : J. B. Lippincott company Pp. 405-429.

6- Atlas R M , Parks L. C. and Brown A E . *Labratory manual of experimental microbiology* (1995): Mosby-year-book, USA

7- Biomeriux company API-Staph system .bioMérieux, 2003; Hazelwood, MO, USA.

8- Hugo, W.B. and Russel, A.D.). *Tube dilution method for MIC,* Pharmaceutical Microbiology (1987) 4th ed. Blackwell scientific Publication.

9- American society for microbiology.. *Instructions to Authors* : Antimicrob. agents chemother(2002) 46: i-xix

10- NCCLS ,*Overview of different breakpoints*(2003) , available on line at www.rivm.nl/earss/Images/breakpointsfinal_tcm61-25277.doc

11- Hiramatsu K, CuiL, Kuroda M, and ItoT. The emergence and evolution of methecillin-resistant Staphylococcus aureus (2001): Trend microbial. 9, 486-493.

12- Kahn S.A ; Nawaz M.S. ; Kahn A.A.and Cerniglia C.E. transfer of Erythromycin resistance from poultry to human clinical strains of Staphylococcus aureus (2000): J. Clinical Microbiology. 5:1832-1838.

13- Al-samaray IE. A study of antibacterial activity of plant extracts on bacterial pathogens isolated from eye infections (2007): Internet journal of microbiology issn: 1937-8289, vol 4 No 1.

14- Gales, A C ; Jones, R N ; Gordon, KA ; Sader, H S ; Wilke, W W ; Beach, M L ; Paller, M A ; and Doern, G V Activity and spectrum of 22 antimicrobial agents against urinary tract infections pathogens isolated from hospitalized patients in Latin America. J. Antimicrob. Chemother. (2000) 45(3):295-303.

15- Rahal J.J. The role of carbapenems in initial therapyfor serios gram negative infections: Critical Care Web. 2008; 12 (suppl. 4) : S5.

16- Al-shakarchi F. Initial therapy for suppurative microbial keratitis in Iraq : Br J Ophthalmol (2007) 91:1583-1587, Vol. 4 No. 1.

17- DeRyke CA, Magilo D and Nicolau DP. Defining theneed for newantimicrobials: clinical and economic implications of resistance in hospitalized patients (2005) Expert Opin Pharmacother 6:837-889.

18- Schlegel L., Sissia G., Fremaux A. and Geslin P. *In vitro* killing activity of combination of β-lactam agents with aminoglycosides against penicillin resistant pneumococci : J. Antimicrobial chemotherapy (1997) 39,95-8.

19- Ambrose P.G. , Bhavani S.M. , Rubino C.M. , Louie A. , Gumbot T. , Forrest A. and Drusano G. L. Pharmacokinetics-pharmacodynamics of antimicrobial therapy: its not just for mice anymore: Clin Ifect Dis. 2007; 44: 79-86.