AJPS, 2010, Vol. 8, No.2

Accepted 29 September 2009

Activity of Meropenem and Ciprofloxacin in vitro Against Oxacillin Resistant Staphylococcus aureus

Suhad Faisal Hatem Al-Mugdadi

Dept. of Medical Microbiology & Biotechnology, College of Pharmacy, University of Al-Mustansiriya.

الخلاصة:

جمعت 150 عينة سريرية لمرضى مراجعين لمستشفى مدينة الطب في بغداد لعزل وتشخيص بكتريا المكورات العنقودية الذهبية Staphylococcus aureus على مستوى النوع باستخدام Api Staph 20 (BioMereux, France)، تم عزل 33 (22%) عزلة من بكتريا المكورات العنقودية الذهبية وقسمت حسب مصدر العزل: (37.5%) 15 عزلة من الجروح، (20%) 10 عزلة من التهاب العظم، (50%) 7 عزلة من مسحات الانف والبلعوم وعزلة واحدة (2%) من الادرار.

تم استخدام 13 مضاداً حيوياً لاختبار الحساسية الدوائية، حيث اتضح ان جميع العزلات كانت مقاومة للبنسلين (100%)، أمبسلين (100%) أما الارثرومايسين فكانت نسبة المقاومة 75.8%، بالنسبة للمضاد الحيوي الاوكساسلين فقد كانت نسبة مقاومة هذه البكتريا له عالية نوعا ما وبنسبة 69.6%، وجد ان اكثر المضادات الحيوية تاثيراً على المكورات العنقودية الذهبية كان الفانكومايسين (100%)، ميروبنيم (100%) وأمبنيم (100%) أما السايبروفلوكساسين والاميكاسين فكانت هذه البكتريا حساسة لها وبنسبة 79% و 76% على التوالي.

تم دراسة Minimum inhibitory concentration (MIC) لكل من المضادين ميروبنيم والسايبروفلوكساسين خارج جسم الكائن الحي لجميع العزلات المقاومة للاوكساسلين (23 عزلة) لمعرفة التاثير التثبيطي ضد المكورات العنقودية الذهبية وعند التراكيز µg / ml (l024-4) حيث كانت نسبة مقاومة المكورات العنقودية الذهبية للميروبنيم 70%عند نقطة التوقف 16 ≤ اما بالنسبة للسايبروفلوكساسين فقد كانت نسبة المقاومة له 74% عند نقطة التوقف 4≤.

Abstract:

A total of 150 clinical specimens were collected from patients attending Medical city Hospital in Baghdad to isolation and identification Staphylococcus aureus on species level by Api staph 20 system (Biomereux , france). 33 (22%) isolates were isolated from Staphylococcus aureus, this isolates divided according to source of isolate: 15 (37.5%) isolate from wounds, 10 (20%) isolate from bone infection, 7 (50%) isolate from nasal and throat swabs, one (2%) isolate from urine.

Accepted 29 September 2009

Thirteen antibiotics were used for sensitivity test; all isolate were resistance to Pencillin (100%), Ampicillin (100%), and while Erythromycin (75.8%). Among the various antimicrobial Oxacillin showed high resisance (69.6%), most effective antibiotics on S. aureus were Vancomycin (100%), Meropenem (100%), while Amikacin and Ciprofloxacin in Percentage 79%, 76% recpectively.

In vitro minimum inhibitory concentration (MIC) of Meropenem and Ciprofloxacin were done to determined inhibition effect against all isolates of S. aureus Which is resist to Oxacillin(23 isolate) in Serial concentration (4-1024) µg / ml, S. aureus was resistant to Meropenem in percentage 70% in break point ≥ 16, while Ciprofloxacim was resistant in percentage 74% in berak point ≥4.

Introduction:

Staphylococci include not only those acquired in hospital but also 80-90% of those isolated in the community. Also, all Staphylococcal strain isolated from clinical samples resistance against various antimicrobial agents (one or more agents), among the resistant pathogens, Methcillin resistant Staphyloccous aureus (MRSA) is one of the major causes of nosocomial infections worldwide and can cause out breaks that are difficult to control[1] .

Soon after admission to hospital, Individuals commonly become contaminated with the hospital flora this has been shown with S. aureus isolates approximately 10% of S. aureus isolates in U.S.A are susceptible to Pencillinase–Stable pencillins Such as Oxacillin and Methicillin. MRSA are resistant to all β-lactam agents, including cephalosporins and carbapenemes[2,3].

Many reports used Oxacillin and Cefoxitin test instead of Methicillin, because it no longer commercially available in U.S.A second, Oxacillin maintion it`s activity during storage better than Methicillin and is more likely to detect hetroresistant strains. In 1990 Oxacillin was chosen as the agent to choice for testing S. aureus resistant to Methicillin[3,4]. Most clinical laboratories U.S.A were used Oxacillin screen agar, disk diffusion, E test, latex agglutination, PCR for detection of MRSA. The result of susceptibility testing by disk diffusion method was very conflicting and this method was similar like other convention Methods such as Oxacillin screen agar, therefore disc diffusion is an easy method for performance in microbiology laboratories for detection of MRSA and the ORSA [5]. One study of Philippine Found that all isolate ORSA on disc diffusion were confirmed as ORSA on E test[6].

Detection of Oxacillin in Staphylococci is important to guide the therapy and prevent the patient from being ulter treat with Vancomycin, which is an antimicrobial agent that presents therapeutic complication and may lead to the selection of resistant mutants [7]. Vancomycin has been the major drug used for treatment of Oxacillin/methicillin-resistant S. aureus infection, but recovery of isolates with intermediate resistance to Vancomycin have spurred the search for

Accepted 29 September 2009

newer agents [8]. Patients with ORSA/MRSA to investigate possible transmission in the hospital environment by nose, skin, wound or burn, long bone leading to necrosis of bone, sputum, CSF, throat and urine [6, 8].

In certain instance of life-threatening infection caused by highly or multiple resistant organisms, the physician may require a quantitative assessment of microorganism susceptibility rather than the qualitative report of sensitive, intermediate, resistant, therefore breakpoint dilution method must use, the concentrations test are chosen carefully to discriminate between susceptible and resistant organisms[9]. The aim of present study was asses activity of Meropenem and Ciprofloxacin *in vitro* against oxacillin–resistant S. aureus.

Materials and Methods:

Samples:

One handered fifty patient attended medical city hospital in Baghdad city during June till August 2008. S. aureus isolates were collected from many clinical source (wound, bone infection, nasal and throat, urine).Typical colonies of Staphylococcus spp. on blood agar were select for further identification:

1- Gram stain.
2- Mannitol salt agar.
3- Catalase test (3% hydrogen peroxide).
4- Coagulase test (rabbit plasma ½ ml mixed with growth of bacteria and Incubated for 1-4h).
5- Api staph 20 system (Bio Merieux, France) [1].

Susceptibility test:

Susceptibility test to antibiotics was determined by the disc diffusion method by Mueller–Hinton agar plate (modified Kerby–Bauer method) [10]. 13 antibiotics were chosen for this study, they belonged to the following growps: Pencillins (Pencillin, Ampicillin, Oxacillin), Cephalosporins (cefotaxim), Aminoglycoside (Gentamycin, Amikacin), Macrolides (Erythromycin), Tetracylines (Tetracyclin), Glycopeptides (Vancomycin), Quinolones (Ciprofloxacin), Antimetabolite (Tri-Methoprim) and carbapenems (Imepenem, Meropenem).

All plates incubated at 37^{o}c for 18-24h except the plate contain Oxacillin disc at 30-35^{o}c, because temperature above 35^{o}c invalidate results of Oxacillin/ Methicillin disc [10].

Accepted 29 September 2009

Antibiotics disc	Code	Concentration	Company
Pencillin	P	10 mcg/disc	Bioanalyse
Ampcillin	A	10 mcg/disc	Himedia
Oxacillin	OX	1mcg/disc	Bioanalyse
Cefotaxim	CTX	10 mcg/disc	Bioanalyse
Gentamycin	G	30 mcg/disc	Bioanalyse
Amikacin	AK	15 mcg/disc	Bioanalyse
Erythromycin	E	30 mcg/disc	Himedia
Tetracyclin	TE	30 mcg/disc	Bioanalyse
Vancomycin	VA	25 mcg/disc	Bioanalyse
Ciprofloxacin	CIP	10 mcg/disc	Bioanalyse
Trimethprim	TR	10 mcg/disc	Himedia
Imepenem	IMP	10mcg/disc	Bioanalyse
Meropenem	MEM	10 mcg/disc	Bioanalyse

Minimum inhibitory concentration (MIC):

To determined MIC for S. aureus used two antibiotics Meropenem and Ciprofloxacin depending on break point for such antibiotics if the value was ≥ from break point that`s mean the microorganism is resistant. To these antibiotics, double dilution agar method was used for MIC by preparing many double diluted (4-1024) µg/ml for Meropenem and Ciprofloxacin[11]. Break point for Meropenem ≥16, while break point for Ciprofloxacin ≥ 4.

Stock solution:

Stock for Meropenem and Ciprofloxacin were prepared by dissolving (0.1) gm in 10 ml sterile D.W[12].

Results and Discussion:

The presence of Staphylococcus aureus in many clinical sources colonized is showed in Table-1. Among 33 (22%) isolate of S. aureus, the high Percentage occur in nasal and throat swab (50%), followed by wound swab (37.5%) and bone infection (20%), while the lowest percentage showed in urine samples (2%) High incidence of S. aureus (48.7%) found in children attending day-care center in Brazil presented the S. aureus only in the Nasal and throat swab [13]. In one study reported that the most common infection of S. aureus in wound (87%) and Nasal and throat swab (80%), while urine was (40%) [8].

In Iran found that the most prevalent in bone infection was S. aureus (55.9%)[14]. While in Al- Basra university teaching hospital Iraq study reported that low incidence of S. aureus (11.8%) in bone infection [15]. Common source of MRSA colonization included nares and wound swab [16]. S. aureus transmitted

Accepted 29 September 2009

by contact with infected person, in addition patients with (ORSA) or (MRSA) transmission in the hospital environment (Nosocomieal infection) and community, therefore many reports recorded different percentage of infection with S. aureus of many kinds of samples, also staff should be screened if there is evidence of continuing transmission in the face of effective physical control measure [4,17].

Table-2 showed that S. aureus isolates resistant to many antimicrobial agents especially to Oxacillin (69.6%), Pencillin (100%), Ampicillin (100%), Erythromycin (75.7%), Trimethoprim (69.6%) and Cefotaxim (69.6%). The most effective drugs on all isolate were Vancomycin (100%), Meropenem (100%) and Imepenem (100%), followed by Ciprofloxacin and Amikacin (79%,76%) respectively. This results agree with one of study in korea, Oxacillin was resist in percentage 68%, Ciprofloxacin 61%, Erythromycin 69%, Gentamycin 66%, Tetracyclin 56% and high resistant to Pencillin (97%) with high sensitive to Vancomycin (100%)[18].

High resistant to Oxacillin (75.2%) and Erythromycin (62.1%) observed in S. aureus strains isolated from many clinical source, among the ORSA strain two strain resistant to Vancomycin and 81% of ORSA have been shown to produce β- loctamase. Vancomycin has been the invasive ORSA infection [8].

In present study was notied that one strain resistant to Oxacillin (1.1%) in Barailian children, while all strain of S. aureus were sensitive to Vancomycin (100%), Imepenem (100%), Amikacin (100%), Ciprofloxacin (100%), while all isolates resistant to Pencillin (100%), Ampcillin (100%) [13]. Many reports found that MRSA/ORSA islates were resistant to Pencillin, Methicillin/Oxacillin, Erythromycin, Gentamycin, Tetracyclin and all isolate sensitive to Vancomycin[19, 20].

The multiresistance to antibiotics may increased with the age of patient, physical contact with infected person in community or acquire in hospitals when they stay for a long hospitalization time [1, 13, 17]. Most of S. aureus isolates which resistant to Methicillin (MRSA) have one plasmide responsible to multiresistance [21].

Table-3 showed that 74% of ORSA isolates were resistant, equal or more break point of Ciprofloxacin ($\geq$ 4), while 70% from isolates were resistant in Break point $\geq$ 16 for Meropenem. In Brazilian hospital survey was found that ORSA isolates were resistance to Ciprofloxacin in percentage 93% in break point $\geq$ 4[22]. In lation America survey was noticed that Meropenem was sensitive (56.2%) in MIC o.o6/>8 µg/ml, while, Ciprofloaxacin was sensitive (58.4%) in MIC 0.5/>2 µg/ml [23]. But in Argentina, ORSA isolates was sensitive to ciprofloxacin (62.8 %) in 0.5/>4 µg/ml [24]. In present study in Iraq that found high resistant (100%) for both antibiotics (CIP, IMP) according to their break point against S. aureus [25].

Accepted 29 September 2009

In our study we tried to determine if new Meropenem and Ciprofloxacin have sufficient in vitro activity against ORSA. We noticed that their difference between results of disc diffusion and MIC Methods against ORSA. This agree with many reports that considered that MIC is the main microbiological parameter used to determined the efficies of antibiotics.

Clinical source	S. aureus isolates	
	+	-
Wounds	15 (37.5%)	25 (62.5%)
Bone infection	10 (20%)	40 (80%)
Nosal +throat swab	7 (50%)	7 (50%)
Urine	1 (2%)	45 (98%)
Total	33 (22%)	117

Table-1: Incidence of S. aureus isolates from many clinical source.

Groups of antibiotics	Code	Resistance	
		No.	%
Pincillins	p	33	100
	A	33	100
	Ox	23	69.6
Cephalosporins	CTX	23	69.6
Aminoglycosides	G	20	60.6
	AK	8	24.2
Macrolides	E	25	75.75
Tetracydines	T	22	66.6
Glycopeptides	V	0	100
Antimetabolite	Tr	23	69.6
Quinolones	CIP	7	21.2
Carbapenems	MEM	0	100
	IMP	0	100

Table-2: Resistance of S. aureus isolates to many groups of antibiotics.

Antimicrobial agents	code	MIC Breakpiont	Resistance No	%	Sensitive No	%
Ciprofloxcin	CIP	≥ 4	17	74%	6	26%
Meropenem	MEM	≥ 16	16	70%	7	30%

Table-3: MIC for CIP& MEM against ORSA according to their break point.

Accepted 29 September 2009

References:

1 - Brook, G. F.; Carrol, K. C.; Butel, J. S. and Morse, S. A. (2007). Medicalmicrobiology. 24th Ed, McGrow- Hill companies U.S.A: 818pp.

2 - Dubey, R.C. and Maheshwari, D.K. (2009). A textbook of microbiology S. chand & company LTD. New Delhi: 912pp.

3 - Centers for disease control and prevention, Laboratory detection of Oxacillin / Methicillin – resistant Staphylococcuc aureus (2007). Cdcinfo @ cdc. Gov.

4 - Bannister, B.; Gillespie, S. and Jones, J. (2006). Infection microbiology and management. 3 rd Ed., Black well published LTD., U.K: 544PP.

5 - Rahbar, M.; Iaghoobi, M. and Fattahi, A. (2006). Comparism of different laboratory method for detection of methicillin resistant Staphylococcus aureus. J. Med. Scie. 22(4):442-445.

6 - Maribel, B.; We, M. D.; Cristina, L.; Crude–pineda, M. D.; Tessa-Tan Torres, M. D.; Cynthia, M.D.; Mediaadoro, C.; Saniel, M.D. and Melecia Antonio–velmonte, M .D. (1999). Nosocomial aqcuistion of OXacillin–resistant Staphylococcus aureus (ORSA) at the Philippine general hospital. phil. J. Microbiol.Infect. Dis., 28 (4): 128-132.

7 - Marshall, S.A.; Pfaller, M. A.; Jones, R.a. (1999). Ability of the vitek card to detect coagulase–negative staphylococci with Mec A and Oxacillin resistant phenotypes. J. clin. Microbiol. 37:2122-2133.

8 - Arslan, S. and zkardes, F. (2007). Slim production and antibiotic susceptibility in staphylococci isolated from clinical samples . Mem. Inst. Oswaldo Cruz. 102(1) 29-33.

9 - Morello, J.A.; Mizer, H.E. and Graanato, P.A. (2006). Laboratory manual and work book in microbiology, 8th Ed., McGraw –Hill companies, New York: Pp: 290.

10 - Vandepitte, J. and Verhaegen, J. (2003). Basic loboratory procedures in clinical bacteriology, 2nd Ed. , World health organization, Geneva : 167pp.

11 - Baron, E.J. and Finegold, S. M. (1990). Paily and Scotts diagnostic Microbiology .8th Ed., Dennis C.Carson (ed) Mosby, Toronto.

12 - NCCLS: Methods for dilution antimicrobial susceptibility test of bacteria that grow aerobically (2002).In approved standard, Wayne, P.A. Nccls.

13 - Bernardo, B.F. and Ueno, M. I(2008). ncidence of Staphylococcus aureus colonization in children attending day – care centers. Rev. Panam Infectol. 10 (1): 20-23.

14 - Mimejad, R.; Fallahi, S.; Kiani, J.; Jeddi, F.; Khoobdel, M.; Jonaidi, N. and Alaeddini, F. (2008). Epidemic assessment of bacterial agents in ostemyelitis and their antibiotic resistance pattern determination. J. Bio. Sci. 8 (2): 478-481.

Accepted 29 September 2009

15 - Mousa, H.A.l.; Hamdan, T. A. and Bakr, S.S. (2001). Clinical and microbiological evaluation of osteomyelitis Bahrain Med. Bull., 23(2):61-65.

16 - Data, R. and Huang, S.S. (2008). Risk infection and death due to methicillin – resistant Staphylococcus aureus in long–Term carriers. Clin. Infec. Dis. 47 (2): 176-181.

17 - Parekh, N. (2007). Methicillin - resistant Staphylococcus aureus- symptoms of MRSA infection. Buzzle. Com.

18 - Lee, K.; Park, K. H.; Jong, S. H.; Lim, H.S.; Shing, J.H.; Young, D.; Ha, G.Y.; Choung, Y. (2006). Futher increase of vancomycin–resistant Enterococcus faecium amikacin and fluoroquinolone–resistant Klebsiella pneumoniae and imepenem–resistant Acinetobacter spp. In Korea: 2003 KONSAR Surveillance. Yonsei Med. J., 47(1):43-54.

19 - Cafferkey, N.T.; Hone, H. and Keane, K. (1985). Antimicrobial chemotherapy of septicemia due to methicillin–resistant. Antimicrob.–agent chemther. 28 (6): 819-823.

20 - Rohain, M.Y.; Roudzah, A.; Lau, M.G.; Zaidatul, A.A.R.; Salbiah, M.N.; Keah, C.; Noraini, A. and Zainuldin, T. (2000). Susceptibilty pattern of Staphylococcus aureus isolated in Malaysian hospital. Inter. J. Antimicrob. Agents. 13(issue 3): 209-213.

21 - Al-Rubaie, S.R.K. (1998). Abacteriological genetical and biochemical study on Staphylococcus aureus protien Aproducer. PH.D. Thesis, Coll. Sci. Univ. Baghdad: Pp: 137.

22 - Sader, H.S.; Goles, A.C.; Pfaller, M. A.; Mendes, R.E.; Zoccoli, C.; Barth, A. and Janes, R.N. (2001). Pathogen frequency and resistant patterns in brazilian hospitals: Summary of results from three years of the SENTRY antimcrobial surveillance program. Braz. J. Infec. Dis., 5(4):200-214.

23 - Sader, H.S.; Jones, R.N.; Gakles, A.C.; Silva, J.B. and pingnatari, A.C. (2000). SENTRY antimicrobial surveillan program report: Latin American and Brazilian results for 1997 through 2001. Braz. J. Infect. Dis. 8 (1): Pp:25-79.

24 - Vargas, L. J.; Casellas , J.M.; Gales, A.; Tom, G.; sader,H.S.;Lanza , A.; Jones, R.N. and Smayeyevsky , J. (2006). Survey of blood stream infection isolates SENTRY antimicrobial surveillance program in Buenos aires, Argentina Rev. Panam. Infectol. 8 (3) :11-17.

25 - Al-Shammary, Z.A.D (2009). Spectum of bacteria causing acute and chronic osteomylitis. M.Sc Thesis, Coll. Med., Univ. Baghdad: Pp: 90.