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In vitro activity of imipenem combination with colistin or rifampicin against clinical isolates of Acinetobacter baumannii and his antimicrobial susceptibility profil

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ABSTRACT: *Background*: Acinetobacter *baumannii* (A. *baumannii*) is one of the most important nosocomial pathogens, Increasing resistance coupled with the fact that few novel antibiotics are currently available or in the pipeline, leaves patients and physicians with a very limited armamentarium against these pathogens. Combination therapies are considered as effective options to overcome this matter. *Materials and methods*: Fifty A. *baumannii* isolates were collected from clinical specimens, from patients in ICU at Ibn Sina Hospital in Rabat, Morocco during the January 2011– January 2012 period. Antimicrobial susceptibilities to amikacin, ceftazidime, ciprofloxacin, amoxcicillin-clavulanic-acid and imipenem were determined by disk diffusion and the E-test method was used to determine antimicrobial susceptibility and the MIC for colistin, imipenem, rifampicin and for the combinations tests. *Results*: 76% of A. *baumannii* isolates were MDR to antibiotics (amoxcicilline + clavulanic acid, imepenem, ciprofloxacin, amikacin, ceftazidim, rifampicin) unless colistin. 100%, 26%,and 14% of isolates were susceptible to colistin, imipenem, and rifampicin, respectively. The MIC50 and MIC90 of imipenem were 24µg/ml and >32 µg/ respectively. The MIC50 and MIC90 of rifampicin were 4 µg/ml and 6 µg/ml, respectively. Imipenem associated with rifampin or with colistin and imipenem alone had the percentages of the rate sensitivity; 28%, 28% and 26%, respectively. *Conclusion*: the findings of this study indicate that colistin has the best activity against A. *baumannii*, whereas imipenem in combination with colistin or rifampicin still a good choice to treat nosocomial infections due to multiresistant A.*baumannii*.

KEYWORDS: Imipenem, colistin, rifampicin, combination, Acinetobacter *baumannii.*

1 INTRODUCTION

Acinetobacter *baumannii* (A. *baumannii*) is one of the most important nosocomial pathogens because of its longevity in the hospital environment and ability to resist various antimicrobial agents, such as resistance to broad-spectrum B-lactam antibiotics by B-lactamases production **[1]**; he has emerged in the last decades as a major cause of healthcare-associated infections and nosocomial outbreaks. **[2]**

The incidence of multidrug-resistant (MDR) A. *baumannii* is increasing worldwide, including Europe, North America, Latin America, and Asia. **[3]**

MDR *A. baumannii* is a rapidly emerging pathogen in healthcare settings, where it causes infections that include bacteremia, pneumonia, meningitis, and urinary tract and wound infections. Antimicrobial resistance poses great limits for therapeutic options in infected patients, especially if the isolates are resistant to the carbapenems. **[2]**

Increasing resistance coupled with the fact that few novel antibiotics are currently available or in the pipeline, leaves patients and physicians with a very limited armamentarium against these pathogens. **[4]**

Combination therapies are considered as effective options to overcome this matter.

Colistin (also called polymyxin E) belongs to the polymyxin group of antibiotics **[5]**. Colistin is a bactericidal drug that binds to lipopolysaccharides and phospholipids in the outer cell membrane of Gram-negative bacteria. It competitively displaces divalent cations from the phosphate groups of membrane lipids, which leads to disruption of the outer cell membrane, leakage of intracellular contents, and bacterial deat. **[6] [7] [34]**

Imipenem is a beta-lactam antibiotic derived from thienamycin with broad spectrum activity used, in combination with cilastin, to treat various infections. Classified as a carbapenem antibiotic. **[9]**

Rifampicin is a bactericidal antibiotic drug of the rifamycin group. It is a semisynthetic compound derived from streptomyces spp that is used as a first line drug for the treatment of tuberculosis worldwide. **[10]**

Antimicrobial agents such as imipenem, colistin and rifampicin have been used for A. *baumannii* treatment. **[3] [11]**

However, colistin-resistant and imipenem-resistant A. *baumannii* have emerged and these isolates are often multidrug-resistant. **[3] [12] [8]**

This is why the combination therapies are considered as effective options to overcome this problem of multiresistance.

The aim of this study was to determine the in vitro activity of colistin, imipenem, and rifampicin alone and in double combinations against A. *baumannii* isolated from patients at Ibn Sina Hospital in Rabat,Morocco.

2 MATERIALS AND METHODS

2.1 BACTERIAL STRAINS

Fifty A. *baumannii* isolates were collected from clinical specimens such as sputum (24), blood (13), urine (11), and pus (2) from patients in ICU at Ibn Sina Hospital in Rabat, Morocco during the January 2011– January 2012 period. Each isolate was collected from a different patient. Bacterial isolation and identification were performed using standard laboratory methods. **[13]**

2.2 ANTIMICROBIAL SUSCEPTIBILITY TESTING AND MIC DETERMINATION

Antimicrobial susceptibilities to amikacin, ceftazidime, ciprofloxacin, amoxcicillin-clavulanic-acid and imipenem were determined by disk diffusion as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines. **[14]**

The E-test method was used to determine antimicrobial susceptibility and the MIC.

Colistin: susceptible ≤2 μg/ml, resistant ≥4 μg/ml; imipenem: susceptible ≤4 μg/ml, resistant ≥16 μg/ml; rifampicin: susceptible ≤2 μg/ml, low-level resistant 4–8 μg/ml and high-level resistant ≥ 256 μg/ml (CLSI, 2011).

A reference strain of Escherichia coli (E. coli) was used as a control strain.

2.3 ANTIMICROBIAL COMBINATION TESTING

The antimicrobial combination tests were made by the E-test method.

E-test strips of the two antimicrobial agents have been placed at an angle of 90 ° on a Mueller Hinton agar plate inoculated with a strain of A. *baumannii*.

The MIC combination is the ihnibition area of each antimicrobial agent cutting the E-test strip.

3 RESULTS

3.1 ANTIMICROBIAL SUSCEPTIBILITY

Our study indicates that 76% of A. *baumannii* isolates were MDR to antibiotics (amoxcicilline + clavulanic acid, imepenem, ciprofloxacin, amikacin, ceftazidim, rifampicin) unless colistin.

Among all the antimicrobial agents tested, 100%, 26%, and 14% of isolates were susceptible to colistin, imipenem, and rifampicin, respectively. The antimicrobial susceptibility of 50 A. *baumannii* isolates against three antimicrobial agents is shown in Table 1.

Table 1: Antimicrobial susceptibility of 50 A. baumannii isolates against Colistin, Imipenem, Rifampicin

Antimicrobial agents	**Number of susceptible strains**	**Rate sensitivity (%)**	**MIC50**	**MIC90**
Colistin	50	100	0.75	2
Imipenem	13	26	24	>32
Rifampicin	7	14	4	6

The MIC50 and MIC90 values of these three antimicrobial agents showed that all isolates wer susceptible to colistin; it had the highest antimicrobial activity (100%).

The MIC50 and MIC90 of imipenem were 24µg/ml and >32 µg/ respectively. The MIC50 and MIC90 of rifampicin were 4 µg/ml and 6 µg/ml, respectively.

ANTIMICROBIAL COMBINATION

The effects of combinations of antimicrobial agents; colistin, imipenem and rifampicin against A. *baumannii* isolates are shown in Table 2.

Table2: Effects of combinations of antimicrobial agents against fifty A.baumannii

Antimicrobial agents	**Number of susceptible strains**	**Rate sensitivity (%)**	**MIC50**
Imipenem	13	26	24
Imipenem+Colistin	14	28	16
Imipenem+Rifampicin	14	28	12

Imipenem associated with rifampin or with colistin and imipenem alone showed remarkable activity against MDR isolates; the percentages of the rate sensitivity was 28%, 28% and 26%, respectively. The results demonstrated that the combination of imipenem and rifampicin reduced the MIC50 of imipenem from 24 to 12µg/ml, and the combination of imipenem and colistin from 24 to 16 µg/ml.

4 DISCUSSION

This study reveals that colistin has a very higher activity against A. *baumannii* rate (100%) that imipenem or rifampicin (Table1).

The same result was obtained from a study done at the University Hospital of Chiang Mai in northern Thailand where it was indicated that colistin has a higher antibacterial activity against A.*baumannii* than imipenem (of 132 isolates of A. *baumannii*, 96% and 64% were susceptible to colistin and imipenem, respectively). **[15]** .

Another study was done at Songklanagarind Hospital in Songkhla Province, confirmed that the Also, the study made in the clinics and intensive care units of Uludağ University Medical School showed that in all studied *A. baumannii* strains ,

susceptibility to colistin was determined to be 100% with E-test methods. **[16]**

Monotherapy based on Colistin showed the problem of nephrotoxicity, neurotoxicity, and a problem of resistance in Gram-negative bacteria. **[17] [18]**

When colistin is administered to patients with a history of chronic renal failure, in this case, nephrotoxicity presents a major health problem. **[19]**

In order to prevent the emergence of resistance to colistin during processing and to reduce the effect of nephrotoxicity, colistin combination therapy may be beneficial.

Our results indicate that 76% of isolates of A. *baumannii* was MDR and 100% of isolates were susceptible to colistin. At roughly the same result was found in China, Greece and Turkey which showed that 84% of isolates of A. *baumannii* strains were MDR and 100% of these strains were susceptible to colistin. **[20] [21] [22]**

This indicates that the colistin was an effective antimicrobial agent against MDR and non-MDR A. *baumannii*.

Imipenem has generally been used in A. *baumannii* treatment **[12].** Our data also affirm that imipenem are good choice for the eradication of A. *baumannii* isolates (MIC50=24).

Based on the results of in vitro studies, rifampicin has been proposed as an alternative antimicrobial agent for the treatment of infections due to A.*baumannii*. **[23] [24] [25]**

While our results show that rifampicin has a low antimicrobial activity against strains of A. *baumannii*, with a rate of sensitivity equal of 14%.

In this study the MIC50 and MIC90 of rifampicin were 4 µg / ml and 6 µg / ml, respectively, to compare results with a study made by GiamarellosBourboulis et al who reported a MIC50 and MIC90 values higher than 2µg/ml. **[11]**

The problem of multiresistance pushes clinicians to consider a combination antimicrobial therapy as an alternative to traiment of infections due to multidrug A.*baumannii*. The presence of synergy could potentially for reduce toxicity and improve outcomes for patients with multiresistant bacteria infection. **[26]**

The combination of imipenem and colistin decreased the MIC50 of imipenem from 24 to 16 µg / ml, which reflects a positive result of the synergy between the two antimicrobial agents, in contrast to another study in Thailand was found no synergistically to combinations of these two antibiotics. **[27]** The same result has been proved after an in vitro study of the combination Colistin / Carbapenem that Is associated with an improvement in MIC, in the majority of the cases, this improvement has suggests synergistic combination or an additive effect. **[28]** .

A study found that the synergy rate between colistin and imipenem was 53.8 µg / ml determined by broth microdilution **[29]** while our study found 28% by the E-test method.

Rifampicin is a hydrophobic antibitique with a large molecular weight, negatively charged, this makes it unable to effectively penetrate only through the outer bacterial membrane of A.*baumannii.* **[30]**

To improve the penetration of rifampicin, it is then combined with other antibiotics which can be related to subtantiels changes in the outer membrane isolates of A. *baumannii*. **[31]**

The degree of sensitivity and specific mechanism of resistance of different A. *baumannii* isolates, are two parameters can influence the effectiveness of rifampicin when combined with another antimicrobial agent. **[30]**

In our study the combination of imipenem and rifampicin decreased the MIC50 of imipenem from 24 to 12 µg / ml, which reflects a very positive result of the synergy between the two antimicrobial agents.

Contrary to a study made by Saballs et al which had results which go against the use of a combination rifampicin / imipenem against isolates of A. *baumannii*.**[32]**

Another study made by Piotr et al confirmed that the use of this combination in the treatment of infections caused by strains of A. *baumannii* with high levels of resistance (MIC> 64 mg / l) is not recommended, by cons, in vitro synergy between rifampicin and imipenem is most likely produced in A. *baumannii* strains with moderate resistance to imipenem (MIC ≤ 64 mg / l). **[33]**

After all these results we suggest that, in patients suffering from MDR A. *baumannii* nosocomial infection that is not responsive to colistin, the combination of imipenem/colistin or imipenem/rifampicin might be beneficial.

5 CONCLUSION

In conclusion, the findings of this study indicate that colistin has the best activity against A. *baumannii*, whereas imipenem in combination with colistin or rifampicin still a good choice to treat nosocomial infections due to multiresistant A.*baumanni*.

LIST OF ABBREVIATIONS

A. *baumannii*: Acinetobacter *baumannii*
CLSI: Clinical Laboratory Standards Institute
E.coli: Escherichia Coli
MDR: Multidrug resistance
MIC: Minimum inhibitory concentration
MIC50: Minimum inhibitory concentration that inhibits 50% of bacterial isolates
MIC90: Minimum inhibitory concentration that inhibits 90% of bacterial isolates

REFERENCES

[1] Wanutsanun Tunyapanit et al. (2014) Antimicrobial susceptibility of Acinetobacter *baumannii* isolated from hospital patients, 10.2306/scienceasia1513-1874.40.028
[2] Matteo Bassetti et al. (2008) Drug Treatment for Multidrug-resistant Acinetobacter *baumannii* Infections, Future Microbiol. 3(6):649-660
[3] Peleg AY, Seifert H, Paterson DL (2008) Acinetobacter *baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 21, 538–82
[4] Russell T et al. Louis C. (2008) Littlefield Celebrating Pharmacy Research Excellence Day
[5] Falagas ME, Kasiakou SK. (2005) Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis; 40:1333.
[6] Kucers A, Crowe S, Grayson ML, Hoy JF. (1997) The use of antibiotics, 5th, Heinemann, London. p.899.
[7] Evans ME, Feola DJ, (1999) Rapp RP. Polymyxin B sulfate and colistin: old antibioticsfor emerging multiresistant gram-negative bacteria. Ann Pharmacother; 33:960.
[8] Li J, Nation RL, Owen RJ, wong S, Spelman D, Franklin C (2007) Antibiograms of multidrug-resistant clinical Acinetobacter baumannii: promising therapeutic options for treatment of infection with colistinresistant strains. Clin Infect Dis 45, 594–8.
[9] MediLexicon International Ltd Bexhill-on-Sea, (2004-2014) UK MediLexicon International Ltd
[10] Eminzade, S., Uras, F., Izzettin, F.V., (2008) Silymarin protects liver against toxic effects of anti-tuberculosis drugs in experimental animals. Nutr. Metab. 5, 18.
[11] Giamarellos-Bourboulis EJ, Xirouchaki E, Giamarellou H (2001) Interactions of colistin and rifampin on multidrug-resistant Acinetobacter baumannii. Diagn Microbiol Infect Dis 40, 117–20.
[12] Jamulitrat S, Thongpiyapoom S, Suwalak N (2007) An outbreak of imipenem-resistant Acinetobacter baumannii at Songklanagarind Hospital: the risk factors and patient prognosis. J Med Assoc Thai 90, 2181–91.
[13] Von Graevenitz A (1995) Acinetobacter, Alcaligenes, Moraxella and other nonfermentative Gram-negative bacteria. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (eds) Manual of Clinical Microbiology, 6th edn, American Society for Microbiology, Washington D.C., pp 520–32
[14] Clinical Laboratory Standards Institute (2011) Performance standards for antimicrobial susceptibility testing; 21st informational supplement, CLSI M100-S21, Clinical and Laboratory Standards Institute Wayne, PA, U.S.A
[15] Leepethacharat K, Oberdorfer P (2007) Acinetobacter baumannii infection and colonization among pediatric patients at Chiang Mai University Hospital. J Infect Dis Antimicrob Agents 24, 63–73
[16] Melda Sınırtaş, Halis Akalın, Suna Gedikoğlu, (2009) Investigation of colistin sensitivity via three different methods in Acinetobacter baumannii isolates with multiple antibiotic resistance, International Journal of Infectious Diseases,Volume 13, Issue 5 ,Pages e217 e220
[17] Li Jian Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, Liolios L (2006) Heteroresistance to colistin in multidrug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother 50, 2946–50.
[18] Pogue JM, Lee J, Marchaim D, Yee V, Zhao JJ, Chopra T, Lephart P, Kaye KS (2011) Incidence of and risk factors for colistin-associated nephrotoxicity in a large academic health system. Clin Infect Dis 53, 879–84.
[19] Michalopoulos AS, Tsiodras S, Rellos K, Mentzelopoulos S, Falagas ME (2005) Colistin treatment in patients with ICU-

acquired infections caused by multiresistant gram-negative bacteria: the renaissance of an old antibiotic. Clin Microbiol Infect 11, 115–21.

[20] Wang H, Chen M (2005) Surveillance for antimicrobial resistance among clinical isolates of gram-negative bacteria from intensive care unit patients in China, 1996 to 2002. Diagn Microbiol Infect Dis 51, 201–8.

[21] Dizbay M, Altuncekic A, Ergut Sezer B, Ozdemir K, Arman D (2008) Colistin and tigecycline susceptibility among multidrug-resistant Acinetobacter baumannii isolated from ventilator-associated pneumonia. Int J Antimicrob Agents 32, 29–32.

[22] Maraki S, Mavros MN, Kofteridis DP, Samonis G, Falagas ME (2012) Epidemiology and antimicrobial sensitivities of 536 multi-drug-resistant gram-negative bacilli isolated from patients treated on surgical wards. Surg Infect 13, 326–31.

[23] Neonakis IK, Spandidos DA, Petinaki E (2011) Confronting multidrug-resistant Acinetobacter baumannii: a review. Int J Antimicrob Agents 37, 102–9.

[24] Timurkaynak F, Can F, Azap OK, Demirbilek M, Arslan H, Karaman SO (2006) In vitro activities of non-traditional antimicrobials alone or in combination against multidrug-resistant strains of Pseudomonas aeruginosa and Acinetobacter baumannii isolated from intensive care units. Int J Antimicrob Agents 27, 224–8.

[25] Tripodi MF, Durante-Mangoni E, Fortunato R, Utili R, Zarrilli R (2007) Comparative activities of colistin, rifampicin, imipenem and sulbactam/ampicillin alone or in combination against epidemic multidrug-resistant Acinetobacter baumannii isolates producing OXA-58 carbapenemases. Int J Antimicrob Agents 30, 537–40.

[26] Marques MB, Brooking ES, Moser SA, Sonke PB, Waites KB (1997) Comparative in vitro antimicrobial susceptibilities of nosocomial isolates of Acinetobacter baumannii and synergistic activities of nine antimicrobial combinations. Antimicrob Agents Chemother 41, 881–5

[27] Wichai Santimaleeworagun, Payom Wongpoowarak, Pantip Chayakul, Sutthiporn Pattharachayakul, Pimpimon Tansakul and Kevin W Garey, (2011) IN VITRO ACTIVITY OF COLISTIN OR SULBACTAM IN COMBINATION WITH FOSFOMYCIN OR IMIPENEM AGAINST CLINICAL ISOLATES OF CARBAPENEMRESISTANT ACINETOBACTER BAUMANNII PRODUCING OXA-23 CARBAPENEMASES, Sciencedirect,Vol 42 No. 4

[28] Ziad Daoud, Najwa Mansour, Khalil Masri, (2013) Synergistic Combination of Carbapenems and Colistin against P. aeruginosa and A. baumanni, OJMM>Vol.3 No.4

[29] G. Leite, C. Kamia Rocha, A. Levin, S. Costa, 783. (2013) In vitro antibiotic synergistic activity against multidrug resistant Acinetobacter baumannii, Session: Poster Abstract Session: Antimicrobials: Treatment of HAI and Resistant Infections*, Room: The Moscone Center: Poster Hall C*

[30] Giannouli M, Popolo AD, Durante-Mangoni E, Bernardo M, Cuccurullo S, Amato G, Tripodi MF, Triassi M, et al (2012) Molecular epidemiology and mechanisms of rifampicin resistance in Acinetobacter baumannii isolates from Italy. Int J Antimicrob Agents 39, 58–63.

[31] Vaara M (1992) Agents that increase the permeability of the outer membrane. Microbiol Rev 56, 395–411.

[32] Saballs M, Pujol M, Tubau F, Peña C, Montero A, Domínguez MA, Gudiol F, Ariza J. (2006) Rifampicin/imipenem combination in the treatment of carbapenem-resistant Acinetobacter baumannii infections, J Antimicrob Chemother.;58(3):697-700.

[33] Piotr Majewski*,Piotr Wieczorek*,Dominika Ojdana,Paweł Tomasz Sacha,Anna Wieczorek & Elżbieta Anna Tryniszewska, (2014) In vitro activity of rifampicin alone and in combination with imipenem against multidrug-resistant Acinetobacter baumannii harboringthe blaOXA-72 resistance gene, Vol. 46, No. 4 , Pages 260-264 (doi:10.3109/00365548.2013.865141)

[33] Li J, Turnidge J, Milne R, et al. (2001) In vitro pharmacodynamic properties of colistin and colistin methanesulfonate against Pseudomonas aeruginosa isolates from patients with cystic fibrosis. Antimicrob Agents Chemother; 45:781