

Antibiotic Combination as Empirical Therapy for Extended Spectrum Beta-Lactamase

Zakariya Al-Muharrmi,¹ Akbar Rafay,¹
Abdullah Balkhair,² Ali A. Jabri¹

Abstract

Background: extended spectrum β -lactamase (ESBL) producing gram negative bacilli are becoming a growing problem worldwide with difficulties in designing a national formulary for empirical treatment of gram negative sepsis.

Objectives: In this study, we investigated the *in vitro* activity of Carbapenems, Piperacillin-Tazobactam, Ciprofloxacin alone or in combination with aminoglycosides against ESBL-producing strains isolated from clinical samples.

Methods: Three hundred and one ESBL-producing *Escherichia coli* and *K. pneumoniae* strains isolated from clinical samples were investigated. Isolates were screened initially for ESBL production using an automated system. All ESBL isolates were further confirmed using the double-disk diffusion method.

Results: The overall Piperacillin-Tazobactam susceptibility was 57.9 (64.4% *E. coli* and 43.6% *Klebsiella pneumoniae*). Only 29.6 % of ESBLs (24.9% *E. coli* and 39.6% *Klebsiella pneumoniae*) were ciprofloxacin susceptible. 98.1% *E. coli* and 93.1% of *Klebsiella pneumoniae* were susceptible to Piperacillin-Tazobactam plus Amikacin combination. 73.7% *E. coli* and 61.4% of *Klebsiella pneumoniae* were susceptible to Piperacillin-Tazobactam plus Gentamicin combination. 96.7% *E. coli* and 91.1% of *Klebsiella pneumoniae* were susceptible to Ciprofloxacin plus Amikacin

combination. 41.2% *E. coli* and 51.5% of *Klebsiella pneumoniae* were susceptible to Ciprofloxacin plus Gentamicin combination.

Conclusion: ESBLs have high resistance profile against Piperacillin/Tazobactam and Ciprofloxacin. The ESBLs from Oman have similar resistance pattern as those reported from UK and USA. This resistance decreases when these drugs are combined with Amikacin. All ESBLs are susceptible to Carbapenems. However, carbapenem overuse can lead to emergence of carbapenem resistant gram negative bacilli and ESBLs. Combination of Amikacin plus Piperacillin/Tazobactam is a feasible empirical therapy for ESBLs

Keywords: extended spectrum β -lactamases (ESBLs), empirical therapy, combination therapy.

Received: 10 August 2007

Accepted: 2 February 2008

From the Departments of ¹Microbiology & Immunology; ²Medicine, College of Medicine and Health Sciences, Sultan Qaboos University, Al Khod, Oman.

Address correspondence and reprint request to: Dr. Zakariya Al Muharrmi, MD, FRCPath, Department of Microbiology & Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, P.O. Box 35, PC 123, Al Khod, Sultanate of Oman.

E-mail: almuharrmi@gmail.com

Introduction

There is a worldwide increase in antimicrobial resistance where most of the most of Gram-negative pathogens are becoming resistant to first-line antibiotics.¹ While there are many mechanisms for antimicrobial resistance among Gram-negative pathogens, the production of extended spectrum β -lactamases (ESBLs) is well documented.² ESBLs were first recognized among *Klebsiella pneumoniae* and *Serratia marcescens* isolates in Germany in 1983 shortly after the introduction of extended spectrum cephalosporins, such as ceftazidime.² By definition, ESBLs are molecular class A or D β -lactamases, which are able to hydrolyze oxyimino cephalosporins at a rate equal to or higher than 10% of that for benzylpenicillin, have an active-site serine, and generally are inhibited by β -lactamase inhibitors such as clavulanic acid, sulbactam, or tazobactam.³

Class A ESBLs belong to three types and these are SHV with more than 60 varieties recognized so far on the basis of unique combinations of amino acid replacements, TEM with more than

135 TEM enzymes currently known, and CTX-M with 40 CTX-M enzymes known so far.⁴ Other uncommon class A ESBLs are BES-1, GES-1, GES-2, IBC-1, IBC-2, PER-1, SFO-1, TLA-1, VEB-1 and VEB-2.⁵ There are at least 12 ESBLs belonging to the OXA type (class D).⁵ Infections caused by ESBLs pose serious challenges to clinicians; because they are resistant to a broad range of β -lactamases, including third-generation cephalosporins, nosocomial infections caused by these organisms complicate therapy and limit treatment options. Moreover, patients infected with ESBLs may have a higher mortality rate and may require longer hospitalization as they are generally sicker and receive more antibiotics than patients who are not infected with ESBL-producing strains.⁶

In this study, we investigated the *in vitro* activity of Carbapenems (Imipenem and Meropenem), Piperacillin-Tazobactam, Ciprofloxacin alone or in combination with aminoglycosides against ESBL-producing strains isolated from clinical samples.

Methods

We have investigated a total of 301 ESBL-producing *Escherichia coli* and *K. pneumoniae* strains isolated from clinical samples. These strains were collected over a period of two years from July 2005 to July 2007 from patients attending Sultan Qaboos University Hospital. Isolates were screened initially for ESBL production using an automated system "Phoenix Identification and Susceptibility System" (by Becton Dickinson, USA, Sparks, MD, USA). It relies on the growth response to selected expanded-spectrum cephalosporins. This test is composed of five wells, each containing a cephalosporin alone or in combination with clavulanic acid (cefepodoxime, ceftazidime, ceftazidime with clavulanic acid, cefotaxime with clavulanic acid and ceftriaxone with clavulanic acid). In this system, the results are also interpreted through a computerized system.⁷ All identified ESBL isolates were further confirmed using the Clinical and Laboratory Standards Institute (CLSI) approved double-disk diffusion method,⁸ which is based on a synergistic increase of inhibition zone of ceftazidime and cefotaxime when they are combined with clavulanate. The test is considered positive when the increase of inhibition zone is (≥ 5 mm).^{7,9,10} Susceptibility results for all ESBL isolates against Ciprofloxacin, Cefepime and Piperacillin-Tazobactam have been tested using the Phoenix Identification and Susceptibility System (Becton Dickinson Biosciences, Sparks, MD). The data analysis and results of antibiotic combinations were conducted using SPSS software.

Results

There was 100% correlation between the BDPhoenix ESBL identification system and the CLSI double disk diffusion confirmatory test. All the 301 isolates were susceptible to Carbapenem (Imipenem & Meropenem). The overall Piperacillin-Tazobactam susceptibility was 57.9% (64.4% *E. coli* and 43.6% *Klebsiella pneumoniae*). Only 29.6% of ESBLs (24.9% *E. coli* and 39.6% *Klebsiella pneumoniae*) were ciprofloxacin susceptible. Cefepime susceptibility was found in 8.7% (9.1% *E. coli* and 0.99% *Klebsiella pneumoniae*). The overall Gentamicin susceptibility was 38.2% (38.3% *E. coli* and 37.6% *Klebsiella pneumoniae*). 94% of ESBLs were susceptible to Amikacin (95.2% *E. coli* and 90.1% *Klebsiella pneumoniae*). 98.1% *E. coli* and 93.1% of *Klebsiella pneumoniae* were susceptible to Piperacillin-Tazobactam plus Amikacin combination. 73.7% *E. coli* and 61.4% of *Klebsiella pneumoniae* were susceptible to Piperacillin-Tazobactam plus Gentamicin combination. 96.7% *E. coli* and 91.1% of *Klebsiella pneumoniae* were susceptible to Ciprofloxacin plus Amikacin combination. 41.2% *E. coli* and 51.5% of *Klebsiella pneumoniae* were susceptible to Ciprofloxacin plus Gentamicin combination.

Discussion

In our study there was 100% correlation between the BDPhoenix ESBL identification system and the CLSI double disk diffusion confirmatory test, the same result was previously reported by Enno Stürenburg et al., where 100% specificity and 100% susceptibility of ("Phoenix Identification and susceptibility system" by Becton Dickinson) in ESBL detection was proved.¹¹ All our isolates were 100% susceptible to Carbapenems (Table 1). It is known that the most successful antibiotic treatment for ESBLs are carbapenems.¹² They are considered the drugs of choice for any serious infections caused by ESBLs.¹³

Piperacillin-Tazobactam susceptibility was variable and demonstrated as 64.4% for *E. coli* and 43.6% for *K. pneumoniae* (Table 1). This is comparable to ESBL susceptibility reported from Europe for Piperacillin-Tazobactam (72.5% *E. coli* and 38.6% *Klebsiella pneumoniae*)¹⁴ (Table 2). Similar susceptibility was reported in USA (80.0% for *E. coli* and 42.1% for *Klebsiella pneumoniae*)¹⁴ (Table 2). The overall susceptibility of ESBLs to piperacillin/tazobactam is only 57.9% (Table 1). This low susceptibility makes piperacillin/tazobactam not a good choice as empirical therapy if suspicion of ESBL is high. However, we found that the some isolates resistant to piperacillin/tazobactam were susceptible to gentamicin and amikacin; and the combined susceptibility to both piperacillin/tazobactam plus an aminoglycoside is additive where piperacillin/tazobactam covers the isolates resistant to aminoglycoside and the aminoglycosides covers the isolates resistant to piperacillin/tazobactam, for example combination of piperacillin/tazobactam plus gentamicin increases the susceptibility to 73.7% against *E. coli* and 61.4% against *K. pneumoniae* (Table 3). The susceptibility is further enhanced if the piperacillin/tazobactam was added with amikacin (susceptibility is 98.1% against *E. coli* and 93.1% against *K. pneumoniae*) (Table 3).

Ciprofloxacin has very low activity against ESBLs, only 29.6% of ESBLs are susceptible (24.9% *E. coli* and 39.6% *K. pneumoniae*). These results are comparable to those in Europe (20.2% *E. coli* and 57.5% *K. pneumoniae*) (Table 3), similar results were also found in USA (20% *E. coli* and 36.8% *K. pneumoniae*)¹⁴ (Table 2). Combination of Ciprofloxacin plus Gentamicin improves the susceptibility to (41.2% for *E. coli* and 51.5% for *K. pneumoniae*). Amikacin boosts the susceptibility of Ciprofloxacin to (96.7% for *E. coli* and 91.1% for *Klebsiella pneumoniae*). Cefepime has very poor activity against ESBLs producing strains; the susceptibility to Cefepime was 9.1% in *E. coli* and 0.99% in *K. pneumoniae*.

It is very clear that Carbapenems are the best empirical therapy for suspected ESBL producing strains. However, repeated

Table 1: Susceptibility of ESBLs to different antibiotics

	<i>E. coli</i> (%)	<i>K. pneumoniae</i> (%)	Overall ESBLs (%)
Carbapenem	100	100	100
Piperacillin-Tazobactam	64.4	43.6	57.9
Ciprofloxacin	24.9	39.6	29.6
Cefepime	9.1	0.99	8.7
Gentamicin	38.3	37.6	38.2
Amikacin	95.2	90.1	94

ESBL: Extended Spectrum Beta - Lactamase

Table 2: ESBL susceptibility in Oman, Europe & USA

	Oman		Europe ¹⁴		USA ¹⁴	
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
Pip-Taz	64.4%	43.6%	72.5%	38.6%	80.0%	42.1%
Cip	24.9%	39.6%	20.2%	57.5	20%	36.8

Pip-Taz: Piperacillin/Tazobactam; Cip: Ciprofloxacin. Europe & USA results adopted from Herman Goossensa et al.¹⁴

Table 3: Susceptibility of ESBLs to Antibiotic Combination

	<i>E. coli</i>	<i>K. pneumoniae</i>
Pip-Taz + AK	98.1	93.1
Pip-Taz + GN	73.7	61.4
CIP + AK	96.7	91.1
CIP + GN	41.2	51.5

Pip-Taz: Piperacillin-Tazobactam; CIP: Ciprofloxacin; Cefe: Cefepime; GN: Gentamicin; AK: Amikacin

exposure to carbapenem has its own cost in terms of emergence of other multi-resistant organisms e.g. *Pseudomonas aeruginosa* and *Acinetobacter* spp.¹⁵ Even though, Robert and coworkers have not detected any increase in carbapenem resistance despite continued use of meropenem and imipenem.¹⁶ The other concern regarding overuse of carbapenem is the emergence of ESBLs tolerant to Carbapenems. Babini and Livermore have seen that of 1% of the ESBL-producing *K. pneumoniae* have decreased susceptibility to meropenem with high MIC values.¹⁷ Kizirgil and coworkers have reported that 5.3% of ESBLs were associated with elevated MICs at 2-4mg/ml of meropenem.¹ In this study 98.4% of ESBLs were susceptible to Imipenem with MIC of $\leq 1\mu\text{g/ml}$ and 99.7% susceptible to Meropenem with MIC of $\leq 1\mu\text{g/ml}$ (Figure 1). The concern about emergence of Carbapenems resistant gram negative bacilli and ESBLs should encourage physicians to look for other alternatives for the empirical treatment of ESBLs.

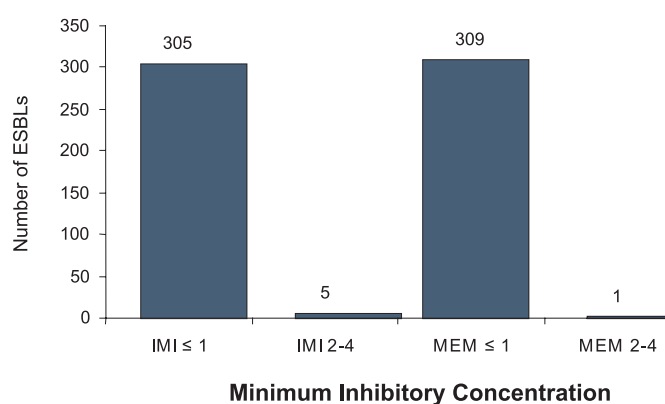


Figure 1: Carbapenem MIC level for ESBLs.

We have demonstrated clearly that Piperacillin/Tazobactam combination with Amikacin has almost similar activity against

ESBL producing *E. coli* (98.1%), to that of Imipenem (98.4%) and Meropenem (99.7%). Piperacillin/Tazobactam combination with Amikacin activity against *K. pneumoniae* (93.1%) is also not far behind that of Imipenem. Ciprofloxacin in combination with Amikacin has an activity against ESBLs (96.7% for *E. coli* and 91.1% for *K. pneumoniae*) comparable to that of Imipenem. The recommendation of combination therapy with Amikacin plus either Piperacillin/Tazobactam or Ciprofloxacin in infections caused by ESBLs is an alternative to Carbapenems as empirical therapy when ESBL infection is suspected.

Conclusion

ESBLs have high resistance profile against Piperacillin/Tazobactam and Ciprofloxacin. The ESBLs from Oman have similar resistance patterns as those reported from United Kingdom and United State of America. This resistance decreases when these drugs are combined with Amikacin. All ESBLs are susceptible to Carbapenems. However, Carbapenem overuse can lead to emergence of carbapenems resistant gram negative bacilli and ESBLs. Combination of Amikacin plus Piperacillin/Tazobactam is a feasible empirical therapy for ESBLs.

References

- Goldstein FW. Combating resistance in a challenging, changing environment. *Clin Microbiol Infect* 2007 Jun;13(Suppl 2):2-6.
- Kotapati S, Kuti JL, Nightingale CH, Nicolau DP. Clinical implications of extended spectrum β -lactamase (ESBL) producing *Klebsiella* species and *Escherichia coli* on cefepime effectiveness. *J Infect* 2005 Oct;51(3):211-217.
- Stürenburg E, Mack D. Extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory, therapy, and infection control. *J Infect* 2003 Nov;47(4):273-295.
- Sorlózano A, Gutiérrez J, de Dios Luna J, Oteo J, Liébana J, Soto MJ, et al. High presence of extended-spectrum β -lactamases and resistance to quinolones in clinical isolates of *Escherichia coli*. *Microbiol Res* 2007;162(4):347-354.
- Jacoby GA, Munoz-Price LS. The new β -lactamases. *N Engl J Med* 2005 Jan;352(4):380-391.
- Ramphal R, Ambrose PG. Extended-spectrum β -lactamases and clinical outcomes: current data. *Clin Infect Dis* 2006 Apr;42(Suppl 4):S164-S172.
- Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum beta-lactamase production in Enterobacteriaceae: review and bench guide. *Clin Microbiol Infect* 2008 Jan;14(Suppl 1):90-103.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Wayne, PA: CLSI, 2005.
- Carter MW, Oakton KJ, Warner M, Livermore DM. Detection of extended-spectrum beta-lactamases in *klebsiellae* with the Oxoid combination disk method. *J Clin Microbiol* 2000 Nov;38(11):4228-4232.
- M'Zali FH, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM. Detection of extended-spectrum beta-lactamases in members of the family enterobacteriaceae: comparison of the MAST DD test, the double disc and the Etest ESBL. *J Antimicrob Chemother* 2000 Jun;45(6):881-885.
- Stürenburg E, Sobottka I, Feucht HH, Mack D, Laufs R. Comparison of BDPhoenix and VITEK2 automated antimicrobial susceptibility test systems for extended-spectrum beta-lactamase detection in *Escherichia coli* and *Klebsiella* species clinical isolates. *Diagn Microbiol Infect Dis* 2003 Jan;45(1):29-34.
- Doménech-Sánchez A, Pascual A, Suárez AI, Alvarez D, Benedi VJ, Martínez-Martínez L. Activity of nine antimicrobial agents against clinical isolates of *Klebsiella pneumoniae* producing extended-spectrum β -lactamases and deficient or not in porins. *J Antimicrob Chemother* 2000 Nov;46(5):858-859.
- Paterson DL, Mulazimoglu L, Casellas JM, Ko WC, Goossens H, Von Gottberg A, et al. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum β -lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 2000 Mar;30(3):473-478.
- Goossens H, Grabein B. Prevalence and antimicrobial susceptibility data for extended-spectrum β -lactamase- and AmpC-producing Enterobacteriaceae from the MYSTIC Program in Europe and the United States (1997-2004). *Diagn Microbiol Infect Dis* 2005 Dec;53(4):257-264.
- Rahal JJ, Urban C, Horn D, Freeman K, Segal-Maurer S, Maurer J, et al. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial *Klebsiella*. *JAMA* 1998 Oct;280(14):1233-1237.
- Masterton RG, Turner PJ. Trends in antimicrobial susceptibility in UK centres: the MYSTIC Programme (1997-2002). *Int J Antimicrob Agents* 2006 Jan;27(1):69-72.
- Babini GS, Livermore DM. Antimicrobial resistance amongst *Klebsiella* spp. collected from intensive care units in Southern and Western Europe in 1997-1998. *J Antimicrob Chemother* 2000 Feb;45(2):183-189.
- Kizirgil A, Demirdag K, Ozden M, Bulut Y, Yakupogullari Y, Toraman ZA. In vitro activity of three different antimicrobial agents against ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* blood isolates. *Microbiol Res* 2005;160(2):135-140.