Prevalence of ESBL and MBL encoding genes in Acinetobacter baumannii strains isolated from patients of intensive care units (ICU)

Marzieh Safari a, Amir Sasan Mozaffari Nejad b,c, Abas Bahador d, Rasool Jafari e, Mohammad Yousef Alikhani a,f,*

a Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
b Young Researchers and Elite Club, Sari Branch, Islamic Azad University, Sari, Iran
c Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
d Department of Microbiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
e Department of Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
f Brucellosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

Received 7 November 2014; revised 30 December 2014; accepted 10 January 2015
Available online 17 January 2015

KEYWORDS
Acinetobacter baumannii; Drug resistance; MBL; ESBL

Abstract The aim of this study was to investigate the prevalence of ESBL and MBL encoding genes among A. baumannii isolates. In this cross sectional study, 100 A. baumannii strains were isolated from ICU wards of 3 educational hospitals of Hamadan City, Iran in 2011. Phenotypic identification of the production of ESBLs and MBLs has been carried out by using E-test and DDST methods, respectively. PCR technique was used for amplification of the ESBL and MBL encoding genes, namely: CTX-M, SHV, TEM, OXA-51, VIM-Family, IMP-Family, SPM-1, SIM-1, and GIM-1. Eighty seven (87%), 95 (95%), 98 (98%) and 95 (95%) out of 100 A. baumannii isolates were resistant to imipenem, meropenem, ceftazidime and cefotaxime, respectively. Also, 99% and 7% of the isolates were MBLs and ESBLs produced phenotypically. Thirty (30%), 20 (20%) and 58 (58%) out of 100 A. baumannii isolates have been confirmed to harbor the blaVIM-family, TEM and SHV genes, respectively. Our results show no significant relationship between the detected gens with production of MBLs and ESBLs in spite of high prevalence of MBL encoding
1. Introduction

Acinetobacter baumannii is an opportunistic pathogen, with the following characteristics of being Gram-negative, oxidase-negative, non-fermentative, nonmotile cocccobacilli and is an unknown natural reservoir and has broad range of antibiotic resistance. The bacterium is prevalent in most places especially in hospitals and other health care institutes (Perez et al., 2007; Yecom et al., 2013; Poirel et al., 2011). Over the last 30 years, Acinetobacter genus has been faced with considerable changes in its taxonomic place (Poleg et al., 2008). High morbidity and mortality are the characteristics of nosocomial infections caused by Acinetobacter spp., which included urinary tract, skin and soft tissue infections, pneumonia and bacteremia especially in patients with severe health conditions (Karageorgopoulos et al., 2008; Safari et al., 2013; Kuo et al., 2012). Most studies conducted by researchers on this bacterium have been carried out concentrating on its drug resistant aspects, which is a major factor limiting the treatment of nosocomial infections (Poirel et al., 2011; Cerqueira and Peleg, 2011). During the last two decades, the advent and widespread dissemination of bacterial infections resistant to beta-lactams, especially to 3rd generation of cephalosporins and carbapenems, has become a globally significant problem (Bush, 2001). Four groups of sorts of powerful enzymes called carbapenemases responsible for antibiotic resistance (Bush, 2001). Four groups of these enzymes have been described in A. baumannii, including IMP-like, SIM-1, NDM-type and VIM-like carbapenemases (Poirel et al., 2011, 2010). Genetic characteristic of MBLs-encoding A. baumannii isolates revealed the presence of blbSIM, blbIMp and blbVIM genes (Poirel et al., 2011). Extended-spectrum ß-lactamases (ESBLs) are encoded by TEM-type, SHV-type and CTX-M-type genes, which are subjected to phenotypical resistance to penicillins and 3rd generation cephalosporins (Pfeifer et al., 2010). Metallo ß-lactamases (MBLs) are sorts of powerful enzymes called carbapenemases responsible for antibiotic resistance (Bush, 2001). Four groups of these enzymes have been described in A. baumannii, including IMP-like, SIM-1, NDM-type and VIM-like carbapenemases (Poirel et al., 2011, 2010). Genetic characteristic of MBLs-encoding A. baumannii isolates revealed the presence of blbSIM, blbIMп and blbVIM genes (Poirel et al., 2011). Extended-spectrum ß-lactamases (ESBLs) are encoded by TEM-type, SHV-type and CTX-M-type genes, which are subjected to phenotypical resistance to penicillins and 3rd generation cephalosporins (Pfeifer et al., 2010). ESBLs are mostly plasmid-mediated and most are parts of the TEM and SHV families of enzymes (Mehrgan and Rahbar, 2008).

One of the notable carbapenem-resistance mechanisms is caused by carbapenem-hydrolyzing ß-lactamases, carbapenemases. In addition, the metallo-ß-lactamases (MßLs) play a crucial role in drug resistance against carbapenems (Poirel and Nordmann, 2006). Also, the extended-spectrum ß-lactamases, ESBLs, play an important role in resistance against later generation cephalosporins such as cefepime, ceftaxime and cefotaxime (Zhaner et al., 2013).

The aim of this study was to investigate the prevalence of ESBLs and MBLs encoding genes and drug resistance against meropenem, imipenem, ceftazidime and cefotaxime among A. baumannii isolates.

2. Materials and methods

2.1. Sampling and isolation of bacteria

The study was a cross sectional study initiated in June 2011 by collecting 100 non-duplicate A. baumannii isolates from clinical specimens from ICU ward patients hospitalized in three educational hospitals of Hamadan City in Iran. The sampling was conducted for 17 months and the isolates were almost from 74 tracheal aspirate, 16 blood, 5 urine, 4 sputum and 1 wound samples. The identification of the isolates has been accomplished by biochemical tests and confirmed by tracking the blaOXA-51-like carbapenemase gene, which is intrinsic to this species, using single PCR (Turton et al., 2006). The confirmed A. baumannii isolates were kept frozen at −70 °C for further tests.

2.2. Antibiogram

Susceptibility to meropenem, imipenem, ceftazidime and cefotaxime (Mast CO, UK) was tested by Kirby-Bauer disk diffusion method. The antibiogram procedure was performed as the manufacturer constructed. In brief, 1.5 × 10⁶ CFU of bacterial suspension, equivalent to McFarland Turbidity Standard No. 0.5, was transferred on Muller-Hinton agar medium (Merck, Germany) and antibiogram disks containing meropenem (10 μg), imipenem (10 μg), cefazidime (30 μg) and ceftaxime (30 μg) were placed on the medium. Then, the media were incubated for 18 h at 35 °C. The results were interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2007). A control strain of Pseudomonas aeruginosa ATCC 27853 was used for quality control of susceptibility testing.

2.3. Phenotypic MBL detection

For determination of phenotypic MßL production among the bacterial isolates, MIC Test Strips (Lioflichem® Italy) containing imipenem and imipenem plus EDTA were used. The E-test procedure was performed according to the manufacturer’s manual. Reduction in the MIC of imipenem of ≥3 dilutions in the presence of EDTA is regarded as a positive result. Also, A. baumannii strain was considered MßL producer if a phenomenon or deformation of the ellipse was obviously observed.

2.4. Phenotypic identification of ESBL producing isolates

Phenotypic identification of ESBL producing isolates have been carried out using DDST screening method. Antibiogram disks containing cefazidime (30 μg), cefotaxime (30 μg), cefazidime (30 μg) + clavulanic acid (10 μg) and cefotaxime (30 μg) + clavulanic (10 μg) were used. Pairs of disks (ceftazidime with cefazidime/clavulanic acid and cefotaxime...
with cefotaxime (clavulanic) were placed on Muller-Hinton agar medium (Merck, Germany) with 20 mm space between them. According to the CLSI criteria and manufacturer instruction, the ≥5 mm inhibition zone of growth in ceftazidime/clavulanic and cefotaxime/clavulanic as cefotaxime and cefotaxime was regarded as an isolate that is producing ESBLs.

2.5. Amplification of blaOXA-51-like gene

The bacterial DNA was extracted by the alkaline lysis method (Kheyrodin and Ghazvinian, 2012). The pair of blaOXA-51 primers (Bioneer® Korea); OXA-F 5'-TAATGCTTGTAGCAGCACCGCTTG-3', and OXA-R 5'-TGGATCGACTCACCTTCATTTGG-3' were used for PCR detection of the genes (Turton et al., 2006). PCR amplification procedure was performed using 25 μl of master mix containing 0.2 μl of Taq polymerase 5 U/μl, 2.5 μl of 10X PCR buffer along with MgCl₂, 1 μl of 10 pM from each reverse and forward primers, 2.5 μl of dNTPs MIX (2 Mm), 3 μl of DNA template, 14.8 μl of DNase-Free and RNase-Free Distilled Water. PCR amplification was done in the thermal cycler device. Agarose gel electrophoresis was done in the thermal cycler device. Agarose gel electrophoresis of the amplified DNA product with 100 bp size marker (Fermentas, Korea) was carried out in 2% agarose gel for 2 h at 80 V and stained with ethidium bromide.

2.7. Amplification of MBL genes

The specific primers (Bioneer® Korea) including VIM-Family, IMP-Family, SPM-1, SIM-1 and GIM-1 (Table 1) were used for PCR amplification of the genes (Ellington et al., 2007). PCR amplification procedure was performed with 25 μl of master mix containing 0.2 μl of Taq polymerase 5 U/μl, 2.5 μl of 10X PCR buffer along with MgCl₂, 1 μl of 10 pM from each reverse and forward primers, 2.5 μl of dNTPs MIX (2 Mm), 3 μl of DNA template, 14.8 μl of DNase-Free and RNase-Free Distilled Water. PCR amplification was done in the thermal cycler device. Agarose gel electrophoresis of the amplified DNA product with 100 bp size marker (Fermentas®, Korea) was carried out in a 2% agarose gel for 2 h at 80 V and stained with ethidium bromide.

2.8. Statistical analysis

Statistical results were calculated by the Statistical Package for the Social Sciences (SPSS Inc., USA) version 16.0 for windows and also, using McNemar and Chi square tests regarding $P \leq 0.05$ as significance level.

3. Results

A. baumannii isolates were confirmed with microbiological and PCR (blaOXA-51) methods and entered to the study (Fig. 1).

3.1. Antibiogram

Susceptibility to meropenem, imipenem, ceftazidime and cefotaxime was evaluated by the Kirby-Bauer disk diffusion

### Table 1 Primmers used for PCR amplification of the studied genes.

| Primer name | Primer sequence (5’ to 3’) | Annealing temp (°C) | Product size (bp) | Reference |
|-------------|---------------------------|---------------------|------------------|-----------|
| CTX-M       | F: TCTTCCAGAATAAGGAATCCC R: CCGTTTCCGCATTACACG | 51 | 909 | Kalai Blagui et al. (2007) |
| SHV         | F: CTTACTCGTICTTATCG R: TCCCGAGATAAATACG | 53 | 868 | Kolar et al. (2010) |
| TEM         | F: ATGAGTATCCACATTCTCCG R: CCAAATCGTATACGTGACG | 53 | 931 | Kalai Blagui et al. (2007) |
| OXA-51      | F: TAATCTGTTGGATCCTGCTTG R: TGG ATTCGACCTCATCTTGG | 52 | 353 | Turton et al. (2006) |
| VIM-Family  | F: GATGGTGTGGGGTGTCGATA R: CAG ATGGCGGACCGACG | 52 | 390 | Ellington et al. (2007) |
| IMP-Family  | F: GGAATAGATGTGGCTTAYTCTC R: CCA AACYACTASGTATATCT | 52 | 188 | Ellington et al. (2007) |
| SPM-1       | F: AAAATCTGGGTAAGGCAAACG R: ACATTATCGCCTGGAACAGG | 54 | 271 | Ellington et al., 2007 |
| SIM-1       | F: TAC AAGGGATTCCGGCATCG R: TAAAGGGCCTGTTCCTCAGTG | 54 | 570 | Ellington et al. (2007) |
| GIM-1       | F: TCG ACACACCCTTGGTCGAA | 54 | 477 | Ellington et al. (2007) |
Eighty seven percent, 95%, 98% and 95% out of 100 *A. baumannii* isolates were resistant to imipenem, meropenem, ceftazidime and cefotaxime, respectively.

### 3.2. Phenotypic MBL detection

For determination of phenotypic MBL production among isolates, MIC Test strips containing imipenem and imipenem along with EDTA were used. Ninety nine percent out of 100 *A. baumannii* isolates were MBL producing.

### 3.3. Phenotypic identification of ESBL producing isolates

Phenotypic identification of ESBL producing isolates have been carried out using DDST screening method. From total of 100 samples, 7% *A. baumannii* isolates identified to produce ESBL enzymes.

### 3.4. Detection of ESBL–SHV, -CTX-M and -TEM genes

Of all 100 *A. baumannii* isolates, 58%, and 20% isolates were harboring HSV and CTX-M genes, respectively. The TEM gene was not found in the studied strains. There was no statistically significant relationship between the presence and absence of HSV and CTX-M genes.

### 3.5. Detection of VIM-Family, IMP-Family, SPM-1, SIM-1 and GIM-1 genes

Thirty percent out of 100 *A. baumannii* isolates has been confirmed to harbor the *bla*<sub>VIM</sub>-family genes (Fig. 2), but the other genes including IMP-Family, SPM-1, SIM-1 and GIM-1 have not been detected (Table 2). No significant relationship was observed between the presence and absence of *bla*<sub>VIM</sub>-family genes in the isolates’ resistance to imipenem and meropenem.

### 4. Discussion

Production of carbapenem-hydrolyzing β-lactamases, also called carbapenemases, is one of the significant mechanisms of carbapenem resistance, in which Methalo β-lactamases (MBLs) possess the principal role in drug resistance against carbapenems (Poirel and Nordmann, 2006). Also, the extended-spectrum β-lactamases, ESBLs, play an important role in resistance against later generation cephalosporins such as cefotaxime, ceftazidime, and cefepime (Zhanel et al., 2013). In this study, the prevalence of ESBLs and MBLs encoding genes and drug resistance against meropenem, imipenem, ceftazidime and cefotaxime among *A. baumannii* isolates has been investigated showing a high resistance rate among the antibiotics.

Totally 100 *A. baumannii* isolates have been examined for 3 ESBLs and 5 MBLs encoding genes. Three out of eight genes have been detected including SHV (58%), TEM (20%) and...
VIM (30%). None of the other studied genes has been detected among 100 isolates of *A. baumannii*, which were isolated from ICU wards of 3 educational Hospitals, Hamadan City, Iran, 2011. Also, no significant relationship has been observed about the presence of the detected ESBL and MBL encoding genes with phenotypical resistance against imipenem, meropenem, cefotaxime and ceftazidime. Eighty seven percent, 95%, 98% and 95% out of 100 *A. baumannii* isolates were resistant to imipenem, meropenem, ceftazidime and cefotaxime, respectively.

The results showed that most of the *A. baumannii* isolates were producing MBLs (99%), but not ESBLs (7%). There was not any significant relationship about phenotypic ESBL and MBL producing and the detected genes. Reports from Iran illustrate the high prevalence of drug resistance and multidrug resistance in *A. baumannii* especially against most effective antibiotics such as imipenem and meropenem (Feizabadi et al., 2008; Peymani et al., 2011). Ting et al. (2013) investigated the drug resistance genes in 7 strains of imipenem-resistant *A. baumannii* including TEM, SHV, CTX-M, DHA, CIT, IMP, VIM, KPC, OXA-23. They detected TEM (100%) and OXA-23 (100%) genes among the isolates, but the other genes such as SHV, CTX-M, DHA, CIT, IMP, VIM, KPC could not be detected from 7 strains of imipenem-resistant *A. baumannii*. In the present study, consistent with Ting et al. (2013), just some of the genes have been detected including SHV (58%), TEM (20%) and VIM (30%). In another study by Shahcheraghi et al. (2011) in Tehran, Iran, they showed that the MBL encoding genes included bla VIM-2, bla SPM-1, bla IMP-2, bla GES-1, bla OXA-51, bla OXA-23 genes among 203 *A. baumannii* isolates. They reported that 6 isolates produce MBLs and 94 isolates produce OXA-type carbapenemase. Their finding suggests that in Tehran the prevalence of MBLs producing *A. baumannii* strains is lower than that of the present study from Hamadan City. They detect *bla*<sub>SPM</sub>-1, *bla*<sub>GES</sub>-1, *bla*<sub>OXA-51</sub>, *bla*<sub>OXA-23</sub> genes among 6, 2, 94 and 84 isolates of the bacterium, respectively (Shahcheraghi et al., 2011).

The previous research by Rezaee et al. (2013) revealed genes coding for IMP, SPM-1, VIM, PER-1, VEB-1, TEM, SHV, GES-1, and CTX-M among 76 Acinetobacter spp. Also, they reported that 37% of isolates carried at least one of the *bla*<sub>PER-1</sub> or *bla*<sub>TEM-1</sub> genes and 13.15% of their studied isolates reported to harbor *bla*<sub>TEM-1</sub> gene, which is similar to that of the present study (20%). Also, none of their studied *A. baumannii* isolates were harboring for *bla*<sub>VEB-1</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M-2</sub> and *bla*<sub>GES-1</sub> (Rezaee et al., 2013). In our study, none of the following genes, CTX-M, IMP, SIM, SPM, and GIM, has been detected among 100 isolates of *A. baumannii*.

Our results show that there was not any significant relationship between the detected genes with production of MBLs and ESBLs. Probably, some other genes rather than what we studied are involved in phenotypic production of MBLs and ESBLs and the subsequent drug resistance in Hamadan, Iran.

5. Conclusion

Despite the high prevalence of phenotypic MBL production and high resistance rate against imipenem and meropenem *A. baumannii* isolates, lower rates of MBL encoding genes have been detected. Also, the high resistance rate against cefazidime and cefotaxime was not in relation with phenotypic and genotypic ESBL production. Probably some other genes rather than what we studied are involved in phenotypic production of MBLs and ESBLs and subsequent high drug resistance in Hamadan, Iran.

Acknowledgments

The authors would like to thank the Vice-chancellor of Research of Hamadan University of Medical Sciences for supporting this project.

References

Bush, K., 2001. New beta-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. Clin. Infect. Dis. 32, 1085–1089.

Cerqueira, G.M., Peleg, A.Y., 2011. Insights into *Acinetobacter baumannii* pathogenicity. IUBMB Life 63, 1055–1060.

Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement. M100-S18. Wayne, PA, 2007.

Ellington, M.J., Kistler, J., Livermore, D.M., Woodford, N., 2007. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J. Antimicrob. Chemother. 59, 321–322.

Feizabadi, M.M., FatollahiAzadeh, B., Taherikalani, M., Rasoolinejad, M., SadeghiFarid, N., Aligholi, M., Soroush, S., MohammediYegane, S., 2008. Antimicrobial susceptibility patterns and distribution of *bla*OXA genes among *Acinetobacter* spp. isolated from patients at Tehran hospitals. Jpn. J. Infect. Dis. 61, 274–278.

Kalai Blagut, S., Achour, W., Abbassi, M.S., Bejaoui, M., Abdelhime, A., Ben Hassen, A., 2007. Nosocomial outbreak of OXA-18-producing *Pseudomonas aeruginosa* in Tunisia. Clin. Microbiol. Infect. 13, 794–800.

Karageorgopoulos, D.E., Kelesidis, T., Kelesidis, I., Falagas, M.E., 2008. Tigecycline for the treatment of multidrug-resistant (including carbapenem-resistant) *Acinetobacter* infections: a review of the scientific evidence. J. Antimicrob. Chemother. 62, 45–55.

Kheryordin, H., Ghazvinian, K., 2012. DNA purification and isolation of genomic DNA from bacterial species by plasmid purification system. Afr. J. Agric. Res. 7, 433–442.

Kolar, M., Bordon, J., Chroma, M., Hricova, K., Stosova, T., Sauer, P., Koukalova, D., 2010. ESBL and AmpC beta-lactamase-producing *Enterobacteriaceae* in poultry in the Czech Republic. Vet. Med. Czech. 55, 119–124.

Kuo, S.C., Chang, S.C., Wang, H.Y., Lai, J.F., Chen, P.C., Shiau, Y.R., Huang1, I.-W., Yang Lauderdale, T.-L., TSAR Hospitals, 2012. Emergence of extensively drug-resistant*Acinetobacter baumannii* complex over 10 years: nationwide data from the Taiwan Surveillance of Antimicrobial Resistance (TSAR) program. In: BMC Infect. Dis. 12, 200.

Mehrgan, H., Rahbar, M., 2008. Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* in a tertiary care hospital in Tehran, Iran. Int. J. Antimicrob. Agents. 31, 147–151.

Peleg, A.Y., Seifert, H., Paterson, D.L., 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin. Microbiol. Rev. 21, 538–582.

Perez, F., Hujer, A.M., Hujer, K.M., Decker, B.K., Rather, P.N., Bonomo, R.A., 2007. Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 51, 3471–3484.

Peymani, A., Nahaei, M.R., Farajnia, S., Hasani, A., Mirsalehian, A., Sohrabi, N., Abbasi, L., 2011. High prevalence of metallo-beta-lactamase-producing *Acinetobacter baumannii* in a teaching hospital in Tabriz, Iran. Jpn. J. Infect. Dis. 64, 69–71.

Pfeifer, Y., Cullik, A., Witte, W., 2010. Resistance to cephalosporins and carbapenems in Gram-negative bacterial pathogens. Int. J. Med. Microbiol. 300, 371–379.
Poirel, L., Bonnin, R.A., Nordmann, P., 2011. Genetic basis of antibiotic resistance in pathogenic Acinetobacter species. IUBMB Life 63, 1061–1067.
Poirel, L., Naas, T., Nordmann, P., 2010. Diversity, epidemiology, and genetics of class D beta-lactamas. Antimicrob. Agents Chemother. 54, 24–38.
Poirel, L., Nordmann, P., 2006. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin. Microbiol. Infect. 12, 826–836.
Yeom, J., Shin, J.H., Yang, J.Y., Kim, J., Hwang, G.S., 2013. H NMR-based metabolite profiling of planktonic and biofilm cells in Acinetobacter baumannii 1656-2. PLoS One 8, e57730.
Rezaee, M.A., Pajand, O., Nahaei, M.R., Mahdian, R., Aghazadeh, M., Ghojazadeh, M., Hojabri, Z., 2013. Prevalence of Ambler class A beta-lactamas and ampC expression in cephalosporin-resistant isolates of Acinetobacter baumannii. Diagn. Microbiol. Infect. Dis. 76, 330–334.
Safari, M., Saidijam, M., Bahador, A., Jafari, R., Alikhani, M.Y., 2013. High prevalence of multidrug resistance and metallo-beta-lactamase (MbetaL) producing Acinetobacter baumannii isolated from patients in ICU Wards, Hamadan, Iran. J. Res. Health Sci. 13, 162–167.
Shahcheraghi, F., Abbasalipour, M., Feizabadi, M., Ebrahimpour, G., Akbari, N., 2011. Isolation and genetic characterization of metallo-beta-lactamase and carbapenemase producing strains of Acinetobacter baumannii from patients at Tehran hospitals. Iran. J. Microbiol. 3, 68–74.
Ting, C., Jun, A., Shun, Z., 2013. Detection of the common resistance genes in Gram-negative bacteria using gene chip technology. Indian J. Med. Microbiol. 31, 142–147.
Turton, J.F., Woodford, N., Glover, J., Yarde, S., Kaufmann, M.E., Pitt, T.L., 2006. Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J. Clin. Microbiol. 44, 2974–2976.
Zhanel, G.G., Lawson, C.D., Adam, H., Schweizer, F., Zelenitsky, S., Lagace-Wiens, P.R., Denisuiak, A., Rubinstein, E., Gin, A.S., et al, 2013. Ceftazidime-avibactam: a novel cephalosporin/beta-lactamase inhibitor combination. Drugs 73, 159–177.