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Will it be a Survivor to Add Clavulanic Acid to Cephalosporins in Order to Overcome Resistance Problem?

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Bacterial resistance is closely associated with the use of antimicrobial agents. Prolonged therapy with antibiotics can lead to the development of resistance in a microorganism that initially is sensitive to antibiotics, but later it can adapt gradually and develop resistance to antibiotics.
Aims: We reviewed whether clavulanic acid plus cephalosporin combinations help to solve the resistance problem.
Methods: We evaluated and reviewed this topic via "Antibiotic Resistance", "Cephalosporin and β-Lactamase Inhibitor Combinations" and our suggestions.
Results: Acquired resistance arises from: (1) mutations in cell genes (chromosomal mutation)

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leading to cross-resistance, (2) gene transfer from one microorganism to other by plasmids (conjugation or transformation), transposons (conjugation), integrons and bacteriophages (transduction). β-Lactamases hydrolyze nearly all β-lactams that have ester and amide bond, e.g., penicillins, cephalosporins, monobactams, andcarbapenems. Serine β-lactamases – cephalosporinases, e.g. AmpC enzyme – are found in *Enterobacter spp*. and *P. aeruginosa* and penicillases in *S. aureus*. Amoxicillin-clavulanate resistance (MIC >16 microg/ml) in *Escherichia coli* is reported previously. Therefore, development of new drugs or combination is necessary for the antimicrobial resistance. To manage the Cephalosporin resistance, Cephalosporin and β-Lactamase Inhibitor Combinations, such as Ceftolozane/tazobactam or Ceftazidime/avibactam have been used.

Conclusion: As resistance to cephalosporins have been increasing, cephalosporin + clavulonate combination will be another choice for managing the antibiotic resistance to the cephalosporins. Our suggestion is based on the success of the clavulonate combination of amoxicillin to manage the antibiotic resistance.

Keywords: Antimicrobial resistance; cephalosporin and β-Lactamase; inhibitor combinations; cephalosporin and clavulonate combination.

1. INTRODUCTION

Development of antibiotics is a target to treat increasingly resistant pathogens [1-3].

The Centers for Disease Control and Prevention (CDC) has recently classified extended-spectrum β-lactamases (ESBLs) and MDR Pseudomonas aeruginosa as serious threats, which are defined as significant antibiotic-resistant threats that have the potential to worsen without ongoing public health monitoring and prevention activities [4]. There are approximately 140 000 health care–associated Enterobacteriaceae infections annually in the United States, and of these, almost 26 000 infections (19%) are caused by ESBL-producing microorganisms [3].

1.1 What is Known about this Topic

Bacterial resistance to antimicrobial drugs is an increasing health and economic problem.

β-lactamases play a role for antimicrobial resistance.

1.2 What this Review Adds

To manage the Cephalosporin resistance, Cephalosporin and β-Lactamase Inhibitor.

Combinations have been used. Ceftolozane/ tazobactam is a new antipseudomonal cephalosporin combined with a well-established β-lactamase inhibitor.

Ceftazidime/ avibactam, another new cephalosporin β-lactamase inhibitor combination.

1.3 Questions for the Experts

- As resistance to cephalosporins have been increasing, cephalosporin + clavulonate combination will be another choice for managing the antibiotic resistance to the cephalosporins
- Our hypothesis is based on the success of the clavulonate combination with amoxicillin to manage the antibiotic resistance.

2. ANTIBIOTIC RESISTANCE

Bacterial resistance to antimicrobial drugs is an increasing health and economic problem [5]. Bacterial resistance is closely associated with the use of antimicrobial agents in clinical practice.

Prolonged therapy with antibiotics can lead to the development of resistance in a microorganism that initially is sensitive to antibiotics, but later adapt gradually and develop resistance to antibiotics [5].

Resistance mechanisms evolved from genes present in organisms that produce antibiotics [6]. Multidrug resistance has been demonstrated *in Pseudomonas aeruginosa (P. aeruginosa), Acinetobacter baumannii (A. baumannii), E. coli* [7] and *Klebsiella pneumoniae 67 (K. pneumoniae),* producing extended-spectrum β-lactamases (ESBL), vancomycin-68 resistant *enterococci, Enterococcus faecium (E. faecium)* (VRE), MRSA, vancomycin-resistant *S. aureus* VRSA, extensively drug-resistant (XDR)

Mycobacterium tuberculosis (M. tuberculosis) [7], *Salmonella enterica* (*S. enterica*) serovar Typhimurium*, Shigella dysenteriae* (*S. dysenteriae*), *Haemophilus influenzae (H. influenzae), Stenotrophomonas,* and *Burkholderia* [8]. Antibiotic resistance can be acquired as a chromosomal mutation, but usually resistance to antibiotics is associated with mobile extrachromosomal DNA elements – plasmids, transposons, and integrons– acquired from other bacteria [7]. Efflux pumps are recognized as the main multidrug resistance mechanism in bacteria [7].

Bacterial resistance to antibiotics can be intrinsic or innate, which is characteristic of a particular bacterium and depends on biology of a microorganism (*E. coli* has innate resistance to vancomycin), and acquired resistance [9]. Acquired resistance occurs from (i) acquisition of exogenous genes by plasmids (conjugation or transformation), transposons (conjugation), integrons and bacteriophages (transduction), (ii) mutation of cellular genes, and (iii) a combination of these mechanisms [6,10-12].

Acquired resistance arises from: (1) mutations in cell genes (chromosomal mutation) leading to cross-resistance, (2) gene transfer from one microorganism to other by plasmids (conjugation or transformation), transposons (conjugation), integrons and bacteriophages (transduction). After a bacterium gains resistance genes to protect itself from various antimicrobial agents, bacteria can use several biochemical types of resistance mechanisms: antibiotic inactivation (interference with cell wall synthesis, e.g., β-lactams and glycopeptide), target modification (inhibition of protein synthesis, e.g., macrolides and tetracyclines; interference with nucleic acid synthesis, e.g., fluoroquinolones and rifampin), altered permeability (changes in outer membrane, e.g., aminoglycosides; new membrane transporters, e.g., chloramphenicol), and “bypass” metabolic pathway (inhibition of metabolic pathway, e.g., trimethoprim-sulfamethoxazole) [5].

Another mechanism of bacterial resistance to antibiotics is specific. Bacteria produce an alternative target (usually an enzyme) that is resistant to inhibition of antibiotic (for example, *MRSA* produces an alternative penicillin-binding proteins (PBP). At the same time, bacteria produce a native target too, which is sensitive to antibiotics [13,14].

Reported mechanisms of resistance to β-lactams such as ceftolozane/tazobactam include β- lactamase production, PBP alteration, upregulation of efflux pumps, and outer membrane porin loss [15,16]. Takeda et al. [17] investigated the effects of β-lactamase production, efflux pump expression, membrane protein deletion, and spontaneous mutations on antimicrobial activity of ceftolozane in comparison with ceftazidime, imipenem, and ciprofloxacin. They found that the MIC90 of ceftolozane was lower than that of the other agents and that it was less susceptible to AmpC β-lactamase than ceftazidime. Ceftolozane’s activity was not affected by efflux pump expression or deletion of the membrane protein OprD, which are known mechanisms of resistance for Pseudomonas aeruginosa. The frequency of selection for spontaneous mutants (6.1× 10-9) for plates containing ceftolozane was lower than that of ceftazidime, imipenem, and ciprofloxacin at concentrations of drug that were 4 times the MIC. However, only imipenem displayed activity against strains of Pseudomonas aeruginosa that produced metallo-β-lactamase [2].

The rates of cross-reactivity between penicillins and cephalosporins are <5% and the rates of cross-reactivity between penicillins and carbapenems are <1%.36 in addition, there is almost no cross-reactivity with aztreonam and β-lactams unless a specific reaction to ceftazidime (which is similar in structure to ceftolozane] exists [18].

2.1 β-Lactamases

The most clinically important β-lactamases are produced by gram- negative bacteria [19] and are coded on chromosomes and plasmids. Genes that encode β- lactamases are transferred by transposons but also they may be found in the composition of integrons [19]. β-Lactamases hydrolyze nearly all β-lactams that have ester and amide bond, e.g., penicillins, cephalosporins, monobactams, and carbapenems. Serine β-lactamases – cephalosporinases, e.g. AmpC enzyme – are found in Enterobacter spp. and *P. aeruginosa* and penicillinases in *S. aureus* [20-24]. Metallo-β-lactamases (MBLs) found in *P. aeruginosa, K. pneumoniae, E. coli*, *Proteus mirabilis* (*P. mirabilis*)*, Enterobacter* spp. have the same role as serine β-lactamases and are responsible for resistance to imipenem, new-generation cephalosporins and penicillins. MBLs

are resistant to inhibitors of β-lactamases but sensitive to aztreonam [20,25].

Inhibition of cell wall synthesis is performed by β-lactams, e.g., penicillins, cephalosporins, carbapenems, monobactams, and glycopeptides, e.g., vancomycin and teicoplanin [5].

3. RESISTANCE TO CEPHALOSPORINS

One of the mechanism of cephalosporin resistance in isolates from both hospital and community settings was the production of CTX-M-type Beta-lactamases (ESBLs), with CTX-M-producing *Escherichia coli* as the most numerous resistant organism overall [26]. Other mechanisms of cephalosporin resistance included production of non-CTX-M ESBLs and AmpC Beta-lactamases. Most ESBL (both CTX-M and non-CTX-M) producers were multiply resistant to non-Beta-lactam antibiotics, including trimethoprim, ciprofloxacin and gentamicin [26]. Conclusions: CTX-M enzymes, which were unrecorded in the UK prior to 2000, have become the major mechanism of cephalosporin resistance in Enterobacteriaceae in South-East England. *E. coli* has overtaken Klebsiella and *Enterobacter* spp. to become the major host for ESBLs. Due to the multiple antibiotic resistance exhibited by many ESBL-producers, these changes have major implications for antimicrobial therapy [26].

Potz et al. [26] reported that over 65% of cephalosporin-resistant *E. coli* harboured ESBLs, and CTX-M ESBLs outnumbered non-CTX ESBLs by more than 10:3 in this species. These proportions were even higher among *Klebsiella* species at 93% and 7:1, respectively.

For all mechanisms of cephalosporin resistance, the mean age of patients was >60 years and most were females. Similar age and sex distributions for patients with ESBL-producing bacteria have been seen in case–control studies investigating ESBL production in *E. coli* and *Klebsiella* spp. in non-hospitalized patients [9,27], and a patient age of over 60 years was shown to be an independent risk factor for infection by ESBL-producing bacteria [9,27].

There is also an increase in ciprofloxacin resistance in recent years in *E. coli, Klebsiella* and *Enterobacter* spp., with a dramatic increase in *E. coli* in particular since 2000 from over 4% in that year to almost 16% in 2004 [28,29].

Multiresistance was common among the organisms with ESBLs, regardless of species and enzyme type. This association is well recognized and is partly because ESBLs are mostly encoded by multiresistance plasmids [30]. Treatment with any of several antibiotics might therefore select for organisms with ESBLs. Recent studies on infections with ESBL-producers outside of hospitals identify prior treatment with cephalosporins, quinolones and penicillins as risk factors, along with recent hospitalization [30].

4. TO MANAGE CEPHALOSPORIN RESISTANCE: CEPHALOSPORIN AND B-LACTAMASE INHIBITOR COMBINATIONS

4.1 Ceftolozane/Tazobactam

Ceftolozane/tazobactam (Zerbaxa, Cubist Pharmaceuticals, Inc), a new antipseudomonal cephalosporin combined with a well-established β-lactamase inhibitor that was developed to target resistant strains of *Pseudomonas aeruginosa* while maintaining low convulsion-inducing activity [31,32]. Ceftolozane was previously known as FR264205 and CXA-101, and the combination of ceftolozane/ tazobactam was formerly known as CXA-201.10, Ceftolozane/tazobactam was approved on December 19, 2014, for the treatment of adults with complicated urinary tract infections (cUTIs) and in combination with metronidazole for the treatment of adults with complicated intra-abdominal infections (cIAIs) [33].

Ceftolozane, like other cephalosporins, is a β-lactam antibiotic that binds penicillin-binding proteins (PBPs) and interferes with peptidoglycan cross-linking of the bacterial cell wall, causing eventual cell lysis [34]. Tazobactam is an irreversible inhibitor of β-lactamase enzymes. Similar to other β-lactamase inhibitors, tazobactam has no antimicrobial activity when used as monotherapy. When given in combination with a β-lactam antibiotic, tazobactam is effective against Gram-negative organisms expressing class A β-lactamases, including narrow-spectrum β-lactamases and ESBLs that are predominantly produced by Enterobacteriaceae [35,36].

Ceftolozane/tazobactam displays bactericidal activity both *in vitro* and *in vivo* activity against *Enterobacter cloacae*, *Escherichia coli*,

Klebsiella oxytoca, K pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Streptococcus anginosus, Streptococcus constellatus, Streptococcus salivarius, and *Bacteroides fragilis*, and it is approved by the Food and Drug Administration (FDA) for the treatment of cIAIs caused by these pathogens when used in combination with metronidazole [32].

Craig and Andes [37] explored the relationship between %T>MIC and the bactericidal activity of ceftolozane dosed in neutropenic mice every 6 hours against 4 strains of *Pseudomonas aeruginosa*, 3 strains of *Escherichia coli,* 5 strains of *K pneumoniae*, and 1 strain of *Enterobacter cloacae*, including 5 strains that produced at least 1 ESBL [37]. Ceftolozane/tazobactam is pregnancy category B, and it is not known whether it is excreted in human milk [32].

4.2 Ceftazidime/Avibactam

Ceftazidime/avibactam, another new cephalosporin β-lactamase inhibitor combination recently approved by the FDA for similar indications, carries the same warning of decreased efficacy in patients with renal impairment [38].

5. CLAVULONIC ACID

Clavulanic acid (Clavulanate) is a suicide inhibitor of bacterial beta-lactamase enzymes from *Streptomyces clavuligerus*. Administered alone, it has weak antibacterial activity against most organisms, but given in combination with beta-lactam antibiotics prevents antibiotic inactivation by microbial lactamase [39]. Pharmacological indication for use with Amoxicillin, clavulanic acid is suitable for the treatment of infections with *Staph. aureus* and *Bacteroides fragilis*, or with beta-lactamase producing *H. influenzae* and *E. coli* [39]. Clavulanic acid, produced by the fermentation of *Streptomyces clavuligerus*, is a beta-lactam structurally related to the penicillins [39]. Clavulanic acid is used in conjunction with amoxicillin for the treatment of bronchitis and urinary tract, skin, and soft tissue infections caused by beta-lactamase producing organisms. Clavulanic acid competitively and irreversibly inhibits a wide variety of beta-lactamases, commonly found in microorganisms resistant to penicillins and cephalosporins [39]. Binding and irreversibly inhibiting the beta-lactamase results in a restoration of the antimicrobial activity of beta-lactam antibiotics against lactamase-secreting-resistant bacteria. By inactivating beta- lactamase (the bacterial resistance protein), the accompanying penicillin/cephalosporin drugs may be made more potent as well. Toxicity related to Clavulanate is gastrointestinal symptoms including stomach and abdominal pain, vomiting, and diarrhea. Rash, hyperactivity, or drowsiness have also been observed in a small number of patients. Potassium Clavulonate is used with amoxicillin in brand names of Amoksilav, Augmentin, Ciblor, Klavocin and Neo- Duplamox [39].

6. OUR SUGGESTION

As resistance to cephalosporins have been increasing, cephalosporin + clavulonate combination will be another choice for managing the antibiotic resistance to the cephalosporins. As written above, Ceftolozane/tazobactam and Ceftazidime/avibactam are also other products developed for antibiotic resistance. Clavulanic acid inhibits a wide variety of beta-lactamases. In penicillin and cephalosporin resistant microorganisms, beta-lactamases are commonly found [39].

Our suggestion is based on the success of the clavulonate combination with amoxicillin to manage the antibiotic resistance. Clavulanic acid (Clavulanate) is a suicide inhibitor of bacterial beta- lactamase enzymes from *Streptomyces clavuligerus*. Clavulonate is used with amoxicillin in brand names of Amoksilav, Augmentin, Ciblor, Klavocin and Neo-Duplamox [39]. Based on this knowledge, we made our suggestion for using cephalosporin and clavulonate together to manage the antimicrobial resistance to the cephalosporins.

Amoxicillin-clavulanate resistance was also reported in Gram negative pathogens [40]. Therefore, development of new drugs or combination is necessary for the antimicrobial resistance. Di Conza et al. [41] reported that for resistance to β-lactam/β-lactamase inhibitors in *Enterobacteria*, ampicillin-sulbactam-resistant isolates remain susceptible to second- and third-generation cephalosporins. When thought for our suggestion, in ampicillin-sulbactam- resistant isolates of *Enterobacteria,* cephalosporin clavulonate combinations should be investigated whether to achieve more success in the treatment.

7. CONCLUSION

As resistance to cephalosporins have been increasing, cephalosporin + clavulonate combination will be another choice for managing the antibiotic resistance to the cephalosporins. As the other combined antibiotics such as Ceftolozane/ tazobactam and Ceftazidime/ avibactam; cephalosporin + clavulonate combination maybe successful for antibiotic resistance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Infectious Diseases Society of America. The 10 × '20 initiative: pursuing a global commitment to develop 10 new antibacterial drugs by 2020. Clin Infect Dis. 2010;50:1081-3.
2. Kollef MH, Golan Y, Micek ST, Shorr AF, Restrepo MI. Appraising contemporary strategies to combat multidrug resistant Gram-negative bacterial infections: proceedings and data from the Gram-negative resistance summit. Clin Infect Dis. 2011;53(S2):S33-55.
3. Sucher AJ, Chahine EB, Cogan P, Fete M. Ceftolozane/Tazobactam: A new cephalosporin and β-lactamase inhibitor combination. Ann Pharmacother. 2015; 49(9):1046-56.
4. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States; 2013.
   Available:http://www.cdc.gov/drugresistance/threat-report-2013/
   (Accessed April 7, 2015)
5. Giedraitienė A, Vitkauskienė A, Naginienė R, Pavilonis A. Antibiotic resistance mechanisms of clinically important bacteria. Medicina (Kaunas). 2011;47(3): 137-46.
6. Hawkey PM. The origins and molecular basis of antibiotic resistance. BMJ. 1998;317:657-60.
7. Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. Cell. 2007;128:1037-50.
8. Džidic S, Šuškovic J, Kos B. Antibiotic resistance mechanisms in bacteria: biochemical and genetic aspects. Food Technol Biotechnol. 2008;46:11-21.
9. Colodner R, Rock W, Chazan B, Keller N, Guy N, Sakran W, Raz R. Risk factors for the development of extended-spectrum β-lactamase-producing bacteria in nonhospitalized patients. Eur J Clin Microbiol Dis. 2004;23:163-7.
10. Rice LB, Sahm D, Binomo RA. Mechanisms of resistance to antibacterial agents. In: Murray PR, Baron EJ, Jorgensen JH, Phaller MA, Yolken RH, editors. Manual of clinical microbiology. Washington: ASM Press. 2003;1074-101.
11. Attacking the enemy: Antimicrobial agents and chemotherapy. In: Mims C, Dockrell HM, Goering RV, Roitt I, Wakelin D, Zuckerman M, editors. Medical microbiology. Elsevier Mosby. 2004;473-507.
12. Raghunath D. Emerging antibiotic resistance in bacteria with special reference to India. J Biosci 2008;33(4):593-603
13. Mayer KH, Opal SM, Medeiros AA. Mechanisms of antibiotic resistance. In: Mandell GL, Bennett JE, Dolin R, editors. Basic principles in the diagnosis and management of infectious diseases. Churchill Livingstone: An Imprint of Elsevier. 1995;212-25.
14. Jacoby GA, Munoz-Price LS. The new β-lactamases. N Engl J Med. 2005; 352:380-91.
15. Goo K, Sim T. Designing new β-lactams: Implications from their targets, resistance factors and synthesizing enzymes. Curr Comput Aided Drug Des. 2011;7:53-80.
16. Drawz SM, Bonomo RA. Three decades of β-lactamase inhibitors. Clin Microbiol Rev. 2010;23:160-201.
17. Takeda S, Nakai T, Wakai Y, Ikeda F, Hatano K. *In vitro* and *in vivo* activities of a new cephalosporin, FR264205, against *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2007;51:826-30.

18. Terico AT, Gallagher JC. Beta-lactam hypersensitivity and cross-reactivity. J Pharm Pract. 2014;27:530-44.
19. Wickens H, Wade P. Understanding antibiotic resistance. Pharm J. 2005;274: 501-4.
20. Thomson JM, Bonomo R. The threat of antibiotic resistance in Gram-negative pathogenic bacteria: β-lactams in peril! Curr Opinion Microbiol. 2005;8:518-24.
21. Bush K, Jacoby GA, Medeiros A. A functional classifi cation scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother. 1995;39(8):1211-33.
22. Garau G, Garcia-Saez I, Bebrone C, Anne C, Mercuri P, Galleni M, et al. Update of the standard numbering scheme for class B β-lactamases. Antimicrob Agents Chemother. 2004;48(7):2347-9.
23. Lambert PA. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. J R Soc Med. 2002;95(Suppl 41):22-6
24. Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. Cell. 2007;128:1037-50
25. Vatopoulos A. High rates of metallo-beta-lactamases-producing *Klebsiella pneumoniae* in Greece – a review of the current evidence. Euro Surveill 2008;13(4):1-6
26. Potz NA, Hope R, Warner M, Johnson AP, Livermore DM. London & South East ESBL Project Group. Prevalence and mechanisms of cephalosporin resistance in *Enterobacteriaceae* in London and South-East England. J Antimicrob Chemother. 2006;58(2):320-6.
27. Rodríguez-Baño J, Navarro MD, Romero L, Martínez-Martínez L, Muniain MA, Perea EJ, Pérez-Cano R, Pascual A. Epidemiology and clinical features of infections caused by extended-spectrum β-lactamase- producing *Escherichia coli* in nonhospitalized patients. J Clin Microbiol. 2004;42:1089-94.
28. Livermore DM, James D, Reacher M, Graham C, Nichols T, Stephens P, Johnson AP, George RC. Trends in fluoroquinolone (ciprofloxacin) resistance in enterobacteriaceae from bacteremias, England and Wales, 1990–1999. Emerg Infect Dis. 2002;8:473-8.
29. Livermore DM. Minimising antibiotic resistance. Lancet Infect Dis. 2005;5: 450-9.
30. Colodner R, Rock W, Chazan B, Keller N, Guy N, Sakran W, Raz R. Risk factors for the development of extended-spectrum β-lactamase-producing bacteria in nonhospitalized patients. Eur J Clin Microbiol Infect Dis. 2004;23:163-7.
31. Toda A, Ohki H, Yamanaka T, Murano K, Okuda S, Kawabata K, Hatano K, Matsuda K, Misumi K, Itoh K, Satoh K, Inoue S. Synthesis and SAR of novel parenteral anti-pseudomonal cephalosporins: Discovery of FR264205. Bioorg Med Chem Lett. 2008;18:4849-52.
32. Zerbaxa (ceftolozane/tazobactam) for injection [prescribing information]. Lexington, MA: Cubist Pharmaceuticals, Inc; 2014.
33. FDA News Release. FDA approves new antibacterial drug Zerbaxa: Fourth new antibacterial drug approved this year. Available:http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm427534.htm (Accessed March 12, 2015)
34. Goo K, Sim T. Designing new β-lactams: Implications from their targets, resistance factors and synthesizing enzymes. Curr Comput Aided Drug Des. 2011;7:53-80.
35. Drawz SM, Bonomo RA. Three decades of β-lactamase inhibitors. Clin Microbiol Rev. 2010;23:160-201.
36. Toussaint KA, Gallagher JC. β-Lactam/β-lactamase inhibitor combinations: From then to now. Ann Pharmacother. 2015;49:86-98.
37. Craig WA, Andes DR. *In vivo* activities of ceftolozane, a new cephalosporin, with and without tazobactam against *Pseudomonas aeruginosa* and *Enterobacteriaceae*, including strains with extended-spectrum β-lactamases, in the thighs of neutropenic mice. Antimicrob Agents Chemother. 2013;57:1577-82.
38. Avycaz® (ceftazidime/avibactam) for injection [prescribing information]. Cincinnati, OH: Forest Pharmaceuticals, Inc; 2015.
39. Clavulanate. Drug Bank. Available:http://www.drugbank.ca/drugs/DB00766 (Accessed online at Sep13, 2015)

40. Leflon-Guibout V, Speldooren V, Heym B, Nicolas-Chanoine M. Epidemiological survey of amoxicillin-clavulanate resistance and corresponding molecular mechanisms in *Escherichia coli* isolates in France: New genetic features of bla(TEM) genes. Antimicrob Agents Chemother. 2000;44(10):2709-14.

41. Di Conza JA, Badaracco A, Ayala J, Rodríguez C, Famiglietti A, Gutkind GO. β-lactamases produced by amoxicillin-clavulanate-resistant enterobacteria isolated in Buenos Aires, Argentina: a new blaTEM gene. Rev Argent Microbiol. 2014;46(3):210-7.

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