Beta-lactamase Inhibitors May Induce Resistance to Beta-lactam Antibiotics in Bacteria Associated with Clinical Infections

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Abstract

The study evaluated in vitro utility of beta-lactamase inhibitors added indiscriminately with most of the therapeutically used beta-lactam antibiotics (BLA). The common trend among clinicians is to use beta-lactams and beta-lactamase inhibitor (BLI) combinations instead of just BLAs because of the vigorously propagated concept that combinations (BLA BLI) have wider antibiotic spectrum and stability than the BLA alone. Data analysis of antimicrobial sensitivity patterns from the clinical epidemiology laboratory (2011-2017) for bacterial isolates from veterinary clinical cases revealed that out of 3233 bacterial isolates 2.8% (91) were tested resistant to BLA BLI discs while they were sensitive to the same BLA used alone. The observation warns against the indiscriminate use of BLA-BLI combinations. Detection of BLI induced resistance to BLAs in Gram-positive, Gram-negative, oxidase positive and oxidase negative bacteria indicated the widespread occurrence of the trait among bacteria. However, further studies are required to understand the mechanism of induction of BLA resistance by BLIs.

Keywords: beta-lactamase inhibitors, beta-lactam antibiotics, Multiple-drug-resistance, Carbapenem resistance

1. Introduction

Clinicians in India and abroad are inclined to use antibiotics almost in every case suspected for infection [1,2]. Among all, beta-lactam antibiotics (BLA) are the most used antibiotics [1]. However, since last several decades, resistance to BLAs is rampant either due to the production of a battery of beta-lactamases or due to mutations in penicillin-binding proteins (PBPs) by the pathogens or due to efflux pump activation [3]. Resistance through the production of beta-lactamases spread rapidly due to transferable extrachromosomal genes [3]. The limited efficacy of BLAs due to bacterial beta-lactamases was overcome by the discovery of beta-lactamase inhibitors (BLI). The most common and commercially available BLIs viz., clavulanic acid, sulfbactam and tazobactam, have little or no direct antimicrobial activity [2]; however, in combination with a BLA, they widen BLA’s antimicrobial spectrum and stability. Class A beta-lactamases as TEM and SHV are inactivated by clavulanic acid, sulfbactam, tazobactam and carbapenems [2-7]. In recent years, resistance to beta-lactam+ beta-lactamase inhibitor combination (IR) has emerged as a new threat for their therapeutic utility. Increasing prevalence of inhibitor-resistant TEM (IRT-1) is detected in Escherichia, Klebsiella, Proteus, Shigella, and Citrobacter species while IR SHVs is common in K. pneumoniae. Besides, non-TEM, non-SHV family IR enzymes like CTX-M, KPC-2, OXA and class B beta-lactamases are also becoming common in bacteria making beta-lactam and beta-lactamase inhibitor combinations useless [2-8].

Though beta-lactamase inhibitors are one of the most widely and commonly used therapeutic agents their adverse interactions with other antibiotics including beta-lactam antibiotics are not known. Now-a-days we know about many ways and mechanisms of induction and spread of antimicrobial drug resistance (AMR) and many more mechanisms are yet to be understood [3]. The biggest quest is, how BLIs may induce BLA
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resistance in a BLA sensitive bacteria? Do BLIs may induce production of IRs or production of variant penicillin binding proteins (PBPs) or induce changes in other outer membrane proteins (OMPs) and their expression, or induce efflux pumps, or there is some antagonistic drug-drug interaction or there is some other mechanism or pathway of action got activated in bacteria. The present study revealed for the first time that BLIs may convert some of the BLAs useless specifically those having good antibacterial activity in absence of BLIs.

2. Materials and Methods

Antibiograms of bacteria (3233) isolated from referred veterinary clinical cases (2011-2017) available in Epidemiology Division of the institute were analysed in Microsoft Excel-2010 to select the strains showing resistance to one or more BLA+BLI combinations but sensitive to the corresponding BLA. To determine sensitivity CLSI criteria [9] was followed where so ever it was applicable. The data available for ampicillin (10g), ampicillin + sulbactam (10 g +10 g), cephalosporin (30 g), ceftazidime + Clavulanic acid (30g +10g), amoxicillin (25g), amoxyycillin + clavulanic acid (30g +10g), amoxicillin + sulbactam (30 g +15 g), cefotaxime (10 g), cefotaxime + clavulanic acid (10g +10g), ceftriaxone (10g), ceftriaxone + tazobactam (30g +10g), piperacillin (100 g), piperacillin + tazobactam (100g +10g) was used to select the target population. All the 91 selected bacteria were revived from the glycerol stocks and retested twice for sensitivity to BLAs and BLA+BLI combinations and minimum inhibitory concentration (MIC) [9,10]. The growth of bacteria around combination discs was resteted for the sensitivity to the corresponding BLA [9,10]. For the selected strains minimum inhibitory concentration (MIC) of BLA and BLIs were determined using E-strips (BioMerieux, India). The data was analysed for finding association between different parameters of antimicrobial drug resistant in different types of bacteria using chi-square/ Fishers exact tests and odds ratio.

3. Results and Discussion

A total of 91 isolates of bacteria belonging to 50 species (Fig 1, Fig 2) had a reduction in the zone of inhibition by 10 mm or more around discs of BLAs in presence of BLIs. Of the 91 bacteria those turned resistant to BLA+BLI combinations, 15 were sensitive (MIC less than or equal to1.0 mgL-1) to all the 7 beta-lactam antibiotics (Fig 1, Fig 2). Sulbactam induced resistance (MIC more than or equal to 4 mg/) to ampicillin in a strain of Acinetobacter lowffii while in 20 strains sensitive to amoxicillin. Tazobactam induced resistant in 4 strains each to ceftriaxone (MIC more than or equal to 2 mg/L) and piperacillin (MIC more than or equal to 8 mg/L). Clavulanic acid induced resistance to ceftazidime (n=22), amoxicillin (n=17), and cefotaxime (n=22) in 61 isolates of bacteria. Except in one isolate of Streptococcus sanguis in none of the isolates both clavulanic acid and sulbactam could induce resistance to BLAs.

Fig. 1: Table. 1a. Resistance to -lactam antibiotics (BLA) in bacteria isolated from veterinary clinical cases and shown induction of resistance to one or more BLAs in presence of -lactamase inhibitors (BLIs).
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Fig. 2: Table. 1b. Resistance to β-lactam antibiotics (BLA) in bacteria isolated from veterinary clinical cases and shown induction of resistance to one or more BLAs in presence of β-lactamase inhibitors (BLIs).

Resistance to BLAs varied significantly among bacteria of different genera viz., Pseudomonas were significantly less often resistant to two BLAs than Escherichia (p, 0.006), Staphylococcus (p, 0.01) and Streptococcus (p, 0.003) strains. Aeromonas strains were more often resistant for four BLAs than Escherichia (p, 0.02), and Streptococcus (p, 0.01) but less often than Pseudomonas (p, 0.04). Resistance for 5 BLAs was more common in pseudomonads (p, 0.03) than in staphylococci.

Both G+O- (p, 0.04) and G+O+ (p, 0.015) bacteria were significantly more often got induced to show resistant for CTX by CLA than G-O- bacteria, while G+O+ bacteria were more often had induced AMX resistance by sulbactam than G-O+ (p, 0.04) and G+O- (p, 0.03) bacteria.

Clavulanic acid induced resistance to CAZ among more aeromonads than staphylococci (p, 0.004) and in more Escherichia than Pseudomonas (p, 0.04) and Staphylococcus (p, 0.002) strains, and among more Streptococcus than Staphylococcus (p, 0.003) strains. Clavulanic acid also induced resistance for CTX more often in pseudomonads than Escherichia (p, 0.005) and staphylococci (p, 0.01). On the other hand, sulbactam induced resistance towards amoxicillin was more often seen in staphylococci than streptococci (p, 0.03) but no difference was noticed in induction of resistance for ceftriaxone and piperacillin among bacteria of different genera.

The β-lactam antibiotics sensitive (MIC less than or equal to 1.5 mg/L) bacteria turning resistant to BLA+BLI combinations do not carry the trait of resistance to next subculture and bacteria remain sensitive to the BLA indicated the transient and temporary nature of the change. Further, the bacteria resistant to a combination...
like cefotaxime + clavulanic acid showing sensitivity to cefotaxime may be sensitive or resistant to amoxicillin + clavulanic or ceftazidime + clavulanic acid and clavulanic acid in combination BLIs with other beta-lactam antibiotics. The observations indicated about drug specific and bacteria specific nature of the trait.

The trait was evident among Gram-positive (Fig. 4, 5), Gram-negative (Fig. 3), oxidase positive (Fig. 6) and oxidase negative (Fig. 3, 4, 5) bacteria isolated from clinical cases. The study indicated the widespread occurrence of the trait among bacteria. Therefore, further studies are required to understand the mechanism and clinical aspect of the beta-lactamase inhibitor-induced beta-lactam antibiotic resistance. Though, several mechanisms of BLA resistance in bacteria are known [2-8]. the one observed in the present study appears to be novel and needs further studies to better understand antimicrobial drug resistance.

**Fig. 4:** Sensitivity of Staphylococcus cohnii to cefotaxime (CTX) which had resistance to cefotaxime + clavulanic acid (CTX-CLA).

**Fig. 5:** Streptococcus porcinus was sensitive to cefotaxime (CTX) but resistance to cefotaxime + clavulanic acid (CTX-CLA), however, equally sensitive to piperacillin tazobactam (PZT) and piperacillin (PIP), resistant to ceftazidime (CAZ) as well as ceftazidime + clavulanic acid (CAZ-CLA).

**Fig. 6:** Pseudomonas aeruginosa sensitivity assay, the strain was resistant to cefotaxime + clavulanic acid (CTX-CLA) but sensitive to cefotaxime (CTX), intermediate sensitive to cefepime (PM, MIC = 3 g/mL) but resistant to cefepime clavulanic acid (PML, MIC > 4 g/mL), sensitive to ceftazidime (TZ, MIC = 1.5 g/mL) and resistant to cefazidime + clavulanic acid (TZL, MIC > 4 g/mL).

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