Original Research Article

Evaluation of Antibiotic Resistance and β- lactamase Production in Clinical Isolates from a Tertiary Care Hospital in Central India

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ABSTRACT

Introduction: Antimicrobial resistance in bacterial pathogens is associated with high morbidity and mortality. We aimed to evaluate antibiotic resistance and β-lactamase production in clinical isolates of a tertiary care hospital in Central India. **Materials and Methods:** Clinical isolates (n=6472 isolates) from patients with infection were identified using standard microbiological techniques. Antibiotic susceptibility testing was performed according to the CLSI guidelines using the Kirby-Bauer disc diffusion technique. AmpC production in *Enterobacteriaceae* isolates was tested in screening test. Cloxacillin combined disc diffusion test was performed using cefoxitin disc with and without cloxacillin. Metallo-β-lactamase production in *Enterobacteriaceae* isolates was tested in screening test. Non-fermenting Gram-negative isolates were tested by combined disc test using imipenem and imipenem-EDTA discs. **Results:** Most bacteremia cases were caused by *Staphylococcus aureus* (43.13%), non-fermenting spp. (27.44%) and coagulase-negative staphylococci (11.76%). *Escherichia coli* (55.85%) was the main cause of urinary tract infection followed by *Acinetobacter* spp. (11.71%) and *Klebsiella pneumoniae* (10.36%). No resistance to linezolid was seen in Gram-positive isolates. Frequency of vancomycin-resistance was about 9% in *Enterococcus* spp. Methicillin resistance was seen in 19% of *S. aureus* isolates. *Enterobacteriaceae* and *Citrobacter freundii* isolates were completely resistant to aminopenicillin, first- and second-generation cephalosporins and cefamycin. Moreover, *Klebsiella* isolates were resistant to aminopenicillin. *Enterobacteriaceae* isolates showed resistance to aminopenicillin (89.87%), cephalosporins (54-90%) and cephamycin (37-45%). *E. coli* isolates were sensitive to piperacillin-tazobactam (87-96%) and imipenem (99.68-100%). Extended spectrum β-lactamase production was seen in 166 *Enterobacteriaceae* isolates (30.24%). AmpC production was seen in 15 (2.73%) *Enterobacteriaceae* isolates. Total β-lactamase production was found in 19.23% of the isolates. The frequency of β-lactamase production was highest in *K. pneumoniae* (51.67%). **Conclusions:** It is necessary to monitor drug resistance and β-lactamase production. Moreover, it is recommended to perform routine β-lactamase testing in microbiology laboratories for determining prevalence of antibiotic resistance and controlling their spread.

KEYWORDS: Antibiotic Resistance, ESBL, β-lactamase, Enterobacteriaceae

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INTRODUCTION

In the last decade, the world has witnessed a dramatic increase in both the proportion and absolute number of multidrug resistant bacterial pathogens. Organizations such as the Centers for Disease Control and Prevention, the European Centre for Disease Prevention and Control and the World Health Organization (WHO) are considering infections caused by multidrug resistant bacteria as an emerging global disease and a major public health problem [1].

Antimicrobial resistance in bacterial pathogens is associated with high morbidity and mortality. More infections caused by resistant microorganisms fail to respond to conventional treatments, and even last-resort

antibiotics are no longer effective. Recently, on the World Health Day, WHO has given the theme ''Combat drug resistance: no action today means no cure tomorrow'' [2].

Resistance to β-lactam antibiotics that are mainstay for treatment is generally due to mobile genes on plasmids for extended spectrum β-lactamase (ESBL), AmpC and carbapenemase production. Since the pipeline of new antibiotic development is nearly dry, surveillance of the resistance and judicious use of available antibiotics is necessary. In this study, we studied antibiotic resistance and β-lactamase production in clinical isolates of a tertiary care hospital in Central India.

MATERIALS AND METHODS

The study was carried out in a tertiary care hospital from January 2016 to June 2016. Clinical isolates from patients with infections were identified using standard microbiological techniques [3]. Antibiotic susceptibility testing was performed according to the CLSI guidelines using the Kirby-Bauer disc diffusion technique. Minimum inhibitory concentration was determined for vancomycin in staphylococcal isolates and for colistin in *Pseudomonas* and *Acinetobacter* isolates [4, 5]. Methicillin resistance in staphylococcal isolates was evaluated by cefoxitin (30 μg) susceptibility testing [4]. ESBL production was evaluated in screening test by observing diameter of inhibition zone for ceftazidime (positive=≤ 22 mm). ESBL production in *Enterobacteriaceae* isolates was evaluated by double disc synergy test (DDST) using amoxiclav (AMC, 20/10 μg), cefotaxime (CTX, 30 μg), piperacillin-tazobactam (PIT, 100/10 μg) and cefepime (CPM, 30μg) discs [6]. In addition to CLSI phenotypic confirmatory test (CPCT), disc potentiation test was also performed using ceftazidime (30 μg), ceftazidime (CAZ) and clavulanic acid (CAC) discs [4].

AmpC production in *Enterobacteriaceae* isolates was tested in screening test (positive= zone diameter of ≤ 18 mm for cefoxitin) and antagonism test for inducible AmpC using cefoxitin (30 μg), cefotaxime (30 μg), ceftazidime (30 μg) and imipenem (10 μg) [6]. Other tests performed included cloxacillin combined disc diffusion test (CCDDT) for both inducible and non-inducible AmpC using cefoxitin (30 μg) disc with and without cloxacillin (200 μg) [6].

Metallo-β-lactamase (MBL) production in *Enterobacteriaceae* isolates was tested in screening test (positive= zone diameter of ≤ 21 mm for meropenem). Non-fermenting Gram-negative isolates were tested by combined disc test using imipenem (10 μg) and imipenem- EDTA (10/750 μg) discs [6].

RESULTS

Among the 6472 isolates tested in the study, 1186 (18.33%) including 1180 bacterial and six Candida isolates showed significant growth. Majority of the bacteremia cases were caused by Staphylococcus aureus (43.13%) followed by non-fermenting spp. (27.44%) and coagulase-negative staphylococci (11.76%). Escherichia coli (55.85%) was the main cause of urinary tract infection followed by Acinetobacter spp. (11.71%) and Klebsiella pneumoniae (10.36%). Enterococcus isolation was about 8%. In skin and soft tissue infections, the most frequent isolates were of Enterobacteriaceae (47.38%), non-fermenters (33.63%) and S. aureus (16.81%). Non-fermenters (63.16%) were also the main cause of lower respiratory tract infection.

No resistance to linezolid was seen in Gram-positive isolates. Vancomycin resistance was about 9% in Enterococcus spp and not seen in staphylococci. Methicillin resistance was seen in 19% of S. aureus isolates. Enterobacteriaceae and Citrobacter freundii isolates were completely resistant to aminopenicillin, first- and second-generation cephalosporins and cefamycin. Moreover, Klebsiella isolates were resistant to aminopenicillin. Enterobacteriaceae isolates showed resistance to aminopenicillin (89.87%), cephalosporins (54-90%) and cephamycin (37-45%). E. coli isolates were sensitive to PIT (87-96%) and imipenem (99.68-100%), although one isolate was

found to be imipenem-resistant. Among aminoglycosides, amikacin (80-91%) was more effective than gentamicin (67-78%), and sensitivity to nitrofurantoin (13-67%) was relatively better. Pseudomonas and Acinetobacter isolates showed high resistance to penicillin (59-85%), third- and fourth-generation cephalosporins (51-99%) and aztreonam (77.68%). These isolates showed sensitivity to PIT (80-92%), imipenem (98.81-99.20%) and colistin (100%).

ESBL production was seen in 166 Enterobacteriaceae isolates (30.24%) in the DDST and CPCT (Table 1).

Table 1. ESBL production in *Enterobacteriaceae* isolates using different methods (n=549)

Test		**DDST (AMC- CTX/ PIT- CPM)**		**Total**
		Positive	Negative	
CPCT (CAZ- CaC)	Positive	158	6	164
	Negative	2	383	385
Total		160	391	549

AmpC production was seen in 15 (2.73%) *Enterobacteriaceae* isolates (Tables 2 and 3).

Table 2. AmpC production in *Enterobacteriaceae* isolates using different tests

Test	**CX- CTX disc antagonism**	**CAZ- IMP disc antagonism**
Number of AmpC-producing isolates	14	15

Table 3. Result of combined AmpC production test and inducible AmpC production test

Test		**Combined AmpC production test**		**Total**
		Positive	Negative	
Inducible AmpC production test	Positive	15	0	15
	Negative	0	534	534
Total		15	534	549

Table 4 shows the results of total β-lactamase production in *Enterobacteriaceae* and non-fermenting isolates.

Table 4. β-lactamase production among *Enterobacteriaceae* and non-fermenting Gram-negative bacilli (n = 967)

Organism	**ESBL**	**AmpC**	**MBL**	**Total**
E. coli (n=316)	70 (22.15)	6 (1.89)	1 (0.32)	77 (24.36)
K. pneumoniae (n=180)	84 (46.67)	9 (5)	0	93 (51.67)
C. freundii (n=31)	6 (19.35)	0	0	6 (19.35)

Proteus spp. (n=22)	6 (27.27)	0	0	6 (27.27)
P. aeruginosa (n=251)	-	-	2 (0.80)	2 (0.80)
Acinetobacter spp. (n=167)	-	-	2 (1.19)	2 (1.19)
Total	166 (30.23)	15 (1.55)	5 (0.52)	186 (19.23)

DISCUSSION

The pattern of antibiotic sensitivity of pathogens in the community and hospitals has been changing. In recent years, antibiotic resistance has become a major health problem worldwide [7]. In the present study, we observed resistance to PIT and carbapenems, the last-resort antibiotics. The spread of carbapenem-resistant bacteria can cause major problems [7]. Methicillin resistance in *S. aureus* could also cause problems in treatment of infections. In the present study, we observed that vancomycin and linezolid are useful for methicillin-resistant *S. aureus*. In addition, vancomycin-resistant enterococci were sensitive to linezolid.

β-lactamase production is the most common mechanism of β-lactam drug resistance in Gram-negative bacteria [8]. *Enterobacteriaceae* bacteria producing both ESBL and AmpC β-lactamases have been increasingly reported worldwide [9]. In the present study, ESBL production was detected amongst *Enterobacteriaceae* isolates using phenotypic methods. Genotypic methods are not available in most clinical microbiology laboratories. The phenotypic methods are simple to perform and interpret. These methods can be performed in routine disc diffusion antibiotic susceptibility testing by adjusting the positions of discs and adding one or two more discs. In CPCT, 164 of 549 *Enterobacteriaceae* isolates produced ESBL. In DDST, ESBL production was found in 160 isolates. The test also detected two additional ESBL producers which were found to be negative in CPCT. This shows that although the tests only slightly differ in sensitivity, it is advisable to use variety of tests. Overall, ESBL production was seen in 166 (30.23%) *Enterobacteriaceae* isolates.

β-lactamase production has been studied in different regions of India. In a study by Bajpai et al. (2017), frequency of ESBL production was 45%, while in study of Nema et al. (2014) and Gupta et al. (2013), it was found to be 48.27% and 52.6%, respectively [10-12]. Thus, frequency of ESBL production may vary depending on the geographical location and time. Method of assessing β-lactamase detection could also affect its frequency.

Chromosomally-mediated β-lactamase production is mainly through expression of *AmpC* gene, which is either constitutive (non-inducible) or inducible. AmpC is inducible in most *Enterobacteriaceae* strains [13]. In the present study, inducible AmpC production was tested amongst *Enterobacteriaceae* isolates using different tests. We found 15 (1.55%) AmpC producers in combined AmpC and inducible AmpC test. It is difficult to conclude whether they produced inducible AmpC alone or along with non-inducible AmpC.

Introduction of MBL or carbapenemase production in non-fermenting Gram-negative bacilli as well as in *Enterobacteriaceae* is of great importance. MBL production was detected in 5 (0.5%) of 967 *Enterobacteriaceae* isolates and non-fermenting Gram-negative bacilli.

In our study, total β-lactamase production was found in 19.23% of the isolates. The frequency of β-lactamase production was highest in *K. pneumoniae* (51.67%). Co-production of β-lactamases was not observed.

CONCLUSION

The widespread use of antimicrobials may be the possible factor responsible for the emergence of resistant strains. It is necessary to monitor drug resistance and β-lactamase production, especially carbapenemase. It is recommended that microbiology laboratories perform β-lactamase testing routinely for determining prevalence of antibiotic resistance, and taking measures to control their spread.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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