Original Research Article

Detection of extended-spectrum beta-lactamases in Pseudomonas aeruginosa and Acinetobacter baumannii and their prevalence in Intensive care unit of a tertiary care hospital

Kaur C.[1], Sharma S.[2], Sharma P.[3]

[1]Dr. Charanjeev Kaur, Assistant Professor, [2]Dr. Sarbjeet Sharma, Professor & Head, [3]Dr. Poonam Sharma, Professor; all authors are affiliated with Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.

Corresponding Author: Dr. Charanjeev Kaur, Assistant Professor, Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India. E-mail: drcharanjeev@gmail.com

Abstract

Introduction: *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have been known to cause variety of infections, among patients admitted in Intensive Care Unit. Non fermenting Gram-negative bacilli are developing resistance to commonly used antibiotics therefore are becoming difficult to treat Among various enzymes produced by bacteria which lead to drug resistance, extended-spectrumbeta-lactamase (ESBL) enzymesis one of the important mechanism of drug resistance. This study that was conducted a) To detect multidrug-resistant *P. aeruginosa* and *A. baumannii* in patients admitted in ICU patients. b) To determine the prevalence of ESBL producing clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter sp.* in the ICU of the tertiary care hospital. **Material and Methods**: The study was performed in the microbiology department of a North Indian rural tertiary care hospital (Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, India) over a period of one year (January 2012 to December 2012). The study included 100 isolates each of *Acinetobacter baumannii & P. aeruginosa.* Identification of both organisms was done using the standard microbiological techniques as described by Colle et al 1996. The antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method. To detect ESBL producing isolates phenotypicaly, Disc approximation test was performed. **Results**: Out of 200 isolates, 100 each of *A. baumannii* and *P. aeruginosa*, weobtained 82 isolates from ICU, 57 & 25 *A. baumannii* and *P. aeruginosa* respectively. Among these 57*A. baumannii* isolates 89.47% isolates were resistant to Ceftazidime and among these 33.33% isolates were ESBL producers.Of 25 *P.aeruginosa* isolates obtained from ICU 84% were found to beresistant to ceftazidime by antibiotic sensitivity testing. Among these 44% were ESBL producers. **Conclusion**: Our results showed high prevalence ofNon fermenting gram negative bacilli in ICU patient's samples, which were multidrug resistant and producers of Extended spectrum Beta lactamase enzymes.

Key words: *Acinetobacter baumannii,* Disc approximation test, ESBL, ICU, *Pseudomonas aeruginosa*

Introduction

Important cause of common infections, occurring in Intensive care units (ICU), like catheter associated urinary tract infections (CAUTI), surgical site infections (SSI), septicemia, are Non fermenting Gram-negative bacilli like *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [1]. Non fermenting Gram-negative bacilli are developing resistance to commonly used antibiotics therefore are becoming difficult to treat because genes coding the resistance determinants gets transferred to them from other resistant organismsand also they are intrinsically resistant to some antibiotics. Bacteria become insusceptible to drugs either when they start producing enzymes which break down the drugs or target sites of drugs in bacteria are alteredor increased efflux of drugs or decreased permeability of porin channels. Among various enzymes produced by bacteria which lead to drug resistance, extended-spectrumbeta-lactamase (ESBL) enzymesare one of the important mechanism of drug resistance. ESBL enzymes act on beta lactam drugs like penicillins, early cephalosporins, and monobactams, and render them inactive, but not on cephamycins and carbapenems.

Clavulanic acid, tazobactam and sulbactam are beta lactamase enzyme inhibitors [2]. Genes coding for production of ESBLenzymes are plasmid based. This plasmid is responsible for the spread of ESBLs among

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various bacterial strains. Multidrug resistant isolates are a matter of serious concern not only for the physicians while treating their patients but are a serious threat for the hospital infection control committee also. ESBL producing *Pseudomonas aeruginosa* and *Acinetobacter sp.* have known to cause outbreaks in hospital settings [3]. Hence, the aim of our study was (a) To identify multidrug- resistant *P. aeruginosa* and *A. baumannii* strains among ICU patients. b) To find the prevalence of *Pseudomonas aeruginosa* and *Acinetobacter sp.* isolates which are ESBL producing,in the ICU of the hospital.

Materialand Methods

The study has been carried out after obtaining the clearance of Institutional ethical committee.

Study design: Prospectivestudy

Study site: Intensive care unit of SGRDIMSR, Amritsar.

Duration of study: January 2012 to December 2012.

Sample size: 100 isolates each of *Acinetobacter baumannii & P. aeruginosa*

Inclusion criteria

Patients admitted in wards during study period were included.
Only *Acinetobacter baumannii & Paeruginosa* isolates were included.

Exclusion criteria

Isolates other than *Acinetobacter baumannii & P.aeruginosa*
Repeat isolates from same patient.

Sample collection and processing: Clinical samples were collected from patients admitted In various wards, according to standard microbiological guidelines [4]. Identification of both the organisms was done using the standard microbiological techniques as described by Colle et al 1996 [5].

Antibiotic sensitivity testing-The susceptibility of the clinical isolates to some routinely used antibiotics was determined on Mueller Hinton agar by the Kirby–Bauer disk diffusion method using Clinical and Laboratory Standards Institute (CLSI) standards [6]. Antimicrobial agents and their disc concentrations used are as follows**;**

Amikacin	30 µg
Ciprofloxacin	5µg
Ceftazidime	30µg
Piperacillin-tazobactam	100/10µg
Imipenam	10µg
Meropenem	10µg
Polymxin B	300 units
Chloramphenicol	5µg
Gentamicin	10µg
Norfloxacin	10µg

The discs were obtained from Hi Media laboratories Pvt Limited

The results were interpreted as per CLSI (CLINICAL LABORATORY STANDARDS INSTITUTE)[6]. *E.coli* ATCC 25922 was used ascontrol organism for antibiotic sensitivity.

Disc approximation method [6]- Isolates found resistant or with decreased susceptibility to Ceftazidime (30µg) third generation cephalosporin antibiotics were subjected to Disc approximation method, a phenotypic test for detection of ESBL production. A disc of Ceftazidime – clavulanic acidand second disc containing Ceftazidime aloneis placed on Mueller hinton agar plate which is inoculated with the test strain, at a distance of 15mm from each other. If zone of

inhibition around ceftazidime- clavulanic aciddiscis ≥5 mmlarger than that around the ceftazidime disc alone was interpreted as confirmatory for ESBL production as per Clinical and Laboratory Standards Institute (CLSI) 201 guidelines [6]. When performing the ESBL confirmatory tests, K. pneumoniae ATCC 700603 and E. coli ATCC 25922 was tested routinely. E. coli ATCC 25922: ≤ 2-mm increase in zone diameter for antimicrobial agent tested alone vs its zone when tested in combination with clavulanic acid was taken as negative control. K. pneumoniae ATCC 700603: ≥ 5-mm increase in ceftazidime clavulanic acid zone diameter was taken as positive control to standardize the test [6].

Test for ESBL Production

Result

Out of total 200 isolates, 100 each of *A. baumannii* and *P. aeruginosa*, 82 isolates were obtained from ICU. Among these 82 isolates, 57 were *A. baumannii* and 25 isolates were *P. Aeruginosa* (Table no:1) followed by 63 isolates from surgery ward 18 & 45 isolates each of *A. baumannii* and *P. aeruginosa* respectively while 16from medicine ward 6 and 10 each of *A . baumannii* and *P. aeruginosa* respectively.

Table No-1: Distribution of A.baumannii & P. aeruginosa isolates in various wards of institute

	A. baumannii	***P. aeruginosa***	**Total**
ICU	57	25	82
Emergency ward	7	5	12
Eye ward	0	2	2
Gynae ward	4	1	5
Medicine ward	6	10	16
Ortho ward	3	9	12
Paed ward	5	3	8
Surgery ward	18	45	63
Total	**100**	**100**	**200**

Table no 2 shows maximum *A. baumannii* isolates resistant to ceftazidime 87%, followed by amino glycosides (Gentamicin 80%, Amikacin 72%), quinolones (Norfloxacin 84.2%, Ciprofloxacin 70%). Susceptibility ofthe isolates was maximum (100%) to Polymyxin B.

Table No-2: Antibiotic Susceptibility Pattern of *A. baumannii & P. aeruginosa*isolated from various wards

Antimicrobials	***A. baumanni***			***P. aeruginosa***		
	Sensitive	**Intermediate**	**Resistant**	**Sensitive**	**Intermediate**	**Resistant**
Amikacin	26	2	72	91	3	6
Gentamicin	19	1	80	52	0	48
Ciprofloxacin	26	4	70	41	0	59
Ceftazidime	13		87	38	1	62
Pipracillin-tazobactum	47	0	53	82	0	17
Imipenem	66	2	32	75	0	25
Meropenem	42	0	58	64	0	36
Chloramphenicol	17	0	64	13	0	69
Norfloxacin	3	0	16	7	0	11
Polymixin-B	100	0	0	100	0	0

As shown in table no 2 maximum*P. aeruginosa*isolates were resistant to chloramphenicol 69%, followed by ceftazidime 62%, quinolones (norfloxacin 61%, ciprofloxacin 59%), aminoglycosides (gentamicin 48%, amikacin 6%), meropenem 36%. No isolate was found to be resistant to Polymyxin B.

Table No-3: Antibiotic Susceptibility Pattern of *A. baumannii & P. aeruginosa*isolated from ICU

Antimicrobials	A. baumannii			P. aeruginosa		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Amikacin	12	2	43	12	2	11
Gentamicin	12	0	45	07	0	18
Ciprofloxacin	16	3	38	05	0	20
Ceftazidime	06	0	51	04	0	21
Pipracillin-tazobactum	24	0	33	18	1	6
Imipenem	35	2	20	15	0	10
Meropenem	23	0	34	09	0	16
Chloramphenicol	12	0	44	05	0	20
Polymixin-B	57	0	0	25	0	0

Distribution of Ceftazidime resistant *A .baumannii* isolates isolated from various specimens in ICU is shown in table 4. Maximum number of isolates 45.61% (26) were obtained from endotracheal tube (ETT) secretions from ICU patients, of which 96.15% (25) were resistant to Ceftazidime, followed by26.3%(15) isolates from pus out of which 86.66% (13) isolates were not susceptible to Ceftazidime.

Table No-4: Distribution of various samples from ICU from which *A. baumannii* was isolated

Sample	*A. baumannii* from ICU	CEFTAZIDIME RESISTANT	ESBL PRODUCER
Endotracheal tube Secretion	26	25(96.15%)	6(23.07%)
Pus	15	13(86.66%)	6(40%)
Blood	10	8(80%)	3(30%)
Sputum	3	3(100%)	3(100%)
Body Fluids	2	1(50%)	0
Urine	1	1(100%)	1(100%)
Total	**57**	**51(89.47%)**	**19(33.33%)**

Out of 26 isolates obtained from ETT Secretions, 6 isolates were to be ESBL producers while 6 isolates from pus were ESBL producers (Table no 4)

In our study number of *P.aeruginosa*isolatesisolatedfrom ICU were 25. (Table No 1) There antibiotic sensitivity test showed 84 % (21) were resistant to ceftazidime (Table no 3).

These ceftazidime resistant isolates when subjected to Disc approximation test showed 44% (11) isolates were ESBL producers (Table no 5)

Table No-5: Distribution of various samples from ICU from which*P.aeruginosa*was isolated.

Sample	*P.aeruginosa* from ICU	Ceftazidime Resistant	ESBL Producer
Endotracheal tube Secretion	11	10(90.9%)	5(45.45%)
Pus	7	7(100%)	4(57.14%)
Urine	5	3(60%)	2(40%)
Blood	1	1(100%)	0
Body fluids	1	0	0
Sputum	0	0	0
Total	**25**	**21(84%)**	**11(44%)**

Maximum number of *P. aeruginosa* isolates 44% (11) were obtained from endotracheal tube secretions in ICU (Table-5), out of which 90.9% (10) were not susceptible to Ceftazidime, followed by 28% (7) isolates obtained from pus samples of ICU patients and all the 7 isolates were resistant to ceftazidime.

Disc approximation test shows that 45.45% (5) isolates from ETT secretions and 57.14% (4) isolates from pus were ESBL producers. (Table no5)

Discussion

Beta-lactamases are classified into fourclasses, to be precise A, B, C, and D, which is based on the amino acids sequence homology. According to Ambler classification A, C, and D classes are called serine-beta-lactamases, and B class beta-lactamases are referred to as MBL [7]. ESBLs are plasmid mediated β-lactamases that confer resistance to broad spectrum β- lactum antibiotics including third and fourth generation cepahlosporins, azetronam, and extended spectrum penicillins.

These plasmids often encode mutations which confere resistance to other broad spectrum agents including aminoglycosides, co-trimoxazole and fluoroquinolones, resulting in organism resistant to most broad spectrum antibiotics [8].

In the present study 87% *A.baumannii* were resistant to ceftazidime, while 72%, 70%, 58% were resistant to Amikacin, Ciprofloxacin, Meropenem respectively. Results were slightly different in the study by Karthik et al 89%, 80%, 72% of *A. baumannii* isolates were resistant to Meropenem, Amikacin & Ciprofloxacin respectively andresistance to Ceftazidime was (36%) [9]. In another study all *Acinetobacter* isolates were 100% resistant to all generations of Cephalosporins & Trimethoprim/ sufamethoxazole and 80-90% resistant to aminoglycosides and beta lactam/ beta lactamase inhibitor combination [10], these are the drugs which are commonlybeing prescribed in the hospital.

However, lesser used antimicrobials like polymyxin B were 100% sensitive. Such observations have also been observed by other investigators wherein susceptibility is attributed to decreased usage of those antimicrobials [11].

Our study revealed (62%) *P.aeruginosa*isolates to be resistant to Ceftazidime while in a previous study by Aggarwal et al resistance to Ceftazidime was 10.35%. Among aminogly cosides, Amikacin showed least resistance, 6% in our study. Similar results were shown in the study by Aggarwal et al [12] While strainsresistant to Gentamicin were 48% in our study, same results were shown by Sarkaret al (45%). We observed (59%) resistance to Ciprofloxacin while in the study of Sarkaret alit was slightly lower i.e (50%)[13].

All the isolates were 100% sensitive to Polymyxin B , same results were also recordedby Aggarwal et al& Sarkar et al[12][13] .

Our study reported very high incidence of ESBL among *P.aeruginosa* from ICU(44%) similar results were observed by Goel *et al*[14]. Butresults by Agarwal et al were different which showed 20.27% of ESBL production [15]. Typical ESBL production was observed in 33.33% among *A.baumannii*in the present study while study by Goel *et al*shows 17.95% prevalence of ESBL and in other studies, ESBL production has been found to range from 20 percent in India to 54.6% in Korea [16].

Another study revealed that 24.3 per cent of NFGNB isolated from ICU patients were ESBLproducers [10]. In this study, Amikacin, Polymyxin B demonstrated maximum sensitivity against NFGNB. Therefore, use of these antibiotics should be restricted to severe infections, especially in critically ill ICU patients, to avoid rapid emergence of resistant strains.

In our study, ESBL producing*P. aeruginosa* and *A. baumannii* were frequently isolated from endotracheal secretions (45.45% and 23.07%, respectively), as also shown by Abd El-Fattah [17].

Conclusion

It can be concluded from the study that *A.baumannii and P.aeruginosa* are emerging as leading hospital pathogens. Resistance to commonly used antibiotics is posing therapeutic challenge to the clinicians. Such strains have been implicated in many recent out breaks mostly in ICUs where extensive use of antibiotics has contributed to the selection of highly resistant strains. The organisms are resistant due to various factors, especially production of ESBL anddetecting them will help in the study of epidemiology of these organisms and hospital infection control programme.

They can obtain resistance determinants and can exist inhospital environment for prolonged period. By performing a simple, easy and economical test i.e disc potentiation test ESBL producing organisms can be diagnosed

A major problem with ESBLs is their capacity to cause therapeutic failure with cephalosporins and azetreonam when host organism appears to be susceptible to these agents in laboratory tests.

Hence, CLSI recommends that laboratories should report ESBL producing isolates as resistant to all penicillins, cephalosporins (including cefepime and cefpirome), and azetreonam irrespective of *in-vitro* test results.

The carbapenems (Ertapenem, Meropenem and Imipenem) are currently considered the drug of choice for serious infections caused by these pathogens. Piperacillin–Tazobactam and Cefoperazone- Sulbactam may be considered options in mild infections and when ESBL producers are demonstrably susceptible *in -vitro*.

Moreover, it is important to implement antibiotic restriction policies to avoid excessive and injudicious use of extended spectrum cephalosporins and Carbapenems in every hospital. Depending upon the antimicrobial resistance testing, antibiotic policy of the hospital isprepared. Drugs like Carbapenems, Polymyxinsare kept as reserve drugs.

Findings: Nil; **Conflict of Interest**: None initiated
Permission from IRB: Yes

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