

Prevalence and Characterisation of Beta Lactamases in Multi Drug Resistant Gram Negative Bacteria Isolated From Intensive Care Units in A Tertiary Care Hospital From Central India: A Phenotypic And Genotypic Study

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Abstract: Antimicrobial resistance is a growing threat worldwide, and a major threat in India where antibiotics are readily available. Multidrug resistant “superbugs are sending millions of people to hospitals around the world . ICUs are good source of infection, where resistance is most observed and which is a major challenge for the intensivists in their clinical practice. Accumulation of resistance traits in Gram negative bacteria has led to multidrug resistance. The major mechanisms of resistance in these Gram negative bacteria are ESBL, AmpC, and carbapenemases production. The present study will be undertaken to determine the spectrum of Gram negative bacteria causing infections in the ICUs and their sensitivity pattern. Also the study will reveal the major resistance mechanisms acquired by these multidrug resistant Gram negative bacteria, which will be detected by phenotypic screening and confirmatory tests and will be confirmed genotypically by Multiplex PCR. These findings will guide the clinicians in appropriate empirical therapy, reduce drug resistance and thus improve patient outcome.

I. Introduction:

Multidrug resistance has been increasing among Gram negative bacteria (Karen Bush,2010).These MDR “superbugs” are increasingly becoming responsible for the increased morbidity and mortality particularly in hospitalised patients.The Intensive care unit is called the epicentre of infections,due to its extremely vulnerable population which are associated with severe clinical conditions along with the impaired immunity,increased risk of becoming infected through multiple procedures and use of invasive devices distorting the anatomical integrity, lapses in infection control practices and indiscriminate use of antibiotics(Nele Brusselaers *et al.*,2011,Loveena Oberoi *et al.*,2013).The incidence of nosocomial (hospital acquired) infections in critically ill patients is much higher than in general ward patients despite the immense advancement in therapeutic technologies (Tawfik *et al.*, 2013).Severe nosocomial infections contribute to prolonged ICU stay,increased morbidity and mortality and increased resource utilisation(Inan *et al.*,2005).The ICU population has the highest occurrence rates of nosocomial infections (20-30%) leading to enormous impact on hospital costs and survival rate. Along with the problem of nosocomial infection goes the burden of “multidrug resistant” bacteria .(Brusselaers *et al.*,2011). Gram negative bacteria represent the most common nosocomial isolates, primarily *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella spp.*, and *Acinetobacter spp.*(Carlos *et al.*,2014). The probability of encountering such a pathogen is far higher in the ICU than in other patient care areas(Tognim *et al.*, 2004).

The Beta lactam antibiotics are the most commonly prescribed antibiotics in ICUs worldwide, which are favoured because of their efficacy, broad spectra and low toxicity(Loveena Oberoi *et al.*,2013).Beta lactam antibiotics are a broad class of antibiotics consisting of agents that contain a beta lactam ring in their molecular structure. These include penicillins, cephalosporins, monobactams and carbapenems(R Lakshmi *et al.*,2014).

The most wide spread cause of resistance to beta lactam antibiotics is the production of enzymes called beta lactamases. Enterobacteriaceae have become one of the most important causes of nosocomial and community acquired infections(Mita Wadekar *et al.*,2013).The rapid and global dissemination of Enterobacteriaceae harbouring plasmid-borne extended spectrum beta lactamases represents a significant clinical threat(Serife *et al.*,2013).The selective pressure which are generated by the indiscriminate use of the beta lactam antibiotics have led to the selection of a variety of mutated forms of beta lactamases such as the ESBLs, AmpC beta lactamases and the metallo beta lactamases which have emerged as the most worrisome resistance mechanism which poses a therapeutic challenge to the health care settings(Deshmukh *et al.*,2011).

Extended spectrum beta lactamases producing organisms confer resistance to penicillin, cephalosporins and monobactams(Paterson *et al.*,2005).Plasmid mediated AmpC beta lactamases confer resistance against penicillins, cephalosporins and monobactams and are not inhibited by commercially available beta lactamase

inhibitors(Philippon et al.,2002,Rodriguez et al.,2003).Carbapenamases are beta lactamases which include serine beta lactamases (KPC, OXA, etc.) and metallo beta lactamases (MBLs) which confer resistance against carbapenems including the other beta lactam antibiotics and beta lactamase inhibitors(Uma Karthika et al.,(2009),K Arunagiri et al.,(2012).

Detection of these enzymes is highly concerned in order to prevent resistant bacteria spread and to treat the infection.At present, there are 2 groups of methods used for detection of these beta lactamases, phenotypic detection which depends on non molecular technique for enzyme detection and genotypic method which depends on molecular technique (K Thirapanmethee, 2012).Phenotypic detection is routinely used in clinical laboratory because it is easy to perform, convenient and inexpensive. However this method does not type ESBL classes also the CLSI (Clinical laboratory standards institute) has not published guidelines for phenotypic detection of AmpC and MBL beta lactamases. Though a number of phenotypic methods have been proposed, the coexistence of diferent classes of beta lactamases in a single bacterial isolate may pose diagnostic and treatment challenges. Hence, it is essential to know accurate prevalence of these beta lactamase producing bacteria particularly from high risk areas like ICU's, so as to formulate an antibiotic policy for giving appropriate empirical therapy in ICU's where the infections caused by MDR Gram negative bacteria is much higher(Loveena Oberoi et al.2013, Khan MKR et al.,2008).

II. Aims And Objectives:

The present study will be done

- a. to identify the Gram negative pathogenic bacteria from the specimens of ICUs
- b. to determine the prevalence rates of multidrug resistant Gram negative bacteria in ICUs by performing the AST and studying their resistance profile.
- c. to determine the different beta lactamases produced by the prevalent Gram negative bacteria by phenotypic methods (ESBL,AmpC,MBL and Carbapenamases) and their co-existence
- d. To detect the beta lactamase genes (ESBL,AMPc and carbapenamases) and their co-existence by genotypic methods.

III. Literature Review:

- 1) Karen Bush 2010, reviewed the role of beta lactamases in antibiotic resistant Gram negative infections, wherein new Beta lactamases that are transferred among species on plasmids with multiple resistance factors are a continuing exercise. It may be possible to limit the effects of these newer beta lactamases by making the epidemiological information available along with judicious use of antibiotics and strict infection control procedures.
- 2) Nele Brusselaers, Dirk Vogelaers and Stijn Blot 2011, concluded that infections due to MDR microorganisms are a rising problem, especially in the ICU where even sensitive pathogens already cause additional morbidity, mortality and hospital costs. Therefore, additional efforts are needed in the future to win this battle. Constant evaluation of current practice on basis of trends in MDR and antibiotic consumption patterns is essential to make progress in this problematic matter.
- 3) Tawfik Abd Motaleb, Mansour I Sayed, Mohammed H Attia, Amal Sharnooby, Mohamed M Farag, Maha Sabaawy 2013, revealed that; age, hospitalization, antibiotic intake, use of mechanical ventilator is all risk factors for colonization by MBL and ESBL producing bacteria in ICU patients. 52.9% patients in ICU were colonized with Gram negative bacteria, out of which 93.4% strains were ESBL producers and 80.4% strains were MBL producers.
- 4) Ravi K.P, Suresh Durairajan, Sankalp Parivar, Ramesh Venkataraman, V. Ramasbramanian and N. Ramakrishnan 2013, concluded that 60% of the cultures from the patients admitted in ICU grew Gram negative organisms with E.coli, Pseudomonas and Acinetobacter species being the commonest isolated pathogens. When choosing empiric antibiotic in acutely ill Indian ICU patients, modifications to western guidelines need to be done using local microbial prevalence and resistance patterns.
- 5) S Dewam, T Sahoo, N Chandra and A Varma 2013, concluded that ESBL producers were the most frequently isolated Gram negative bacterial isolates in the ICU of tertiary care hospital in North India. In view of significant prevalence of multidrug resistance amongst Gram negative organisms in the ICU, regular surveillance of antibiotic susceptibility patterns plays a crucial role for setting orders to guide the clinician in choosing empirical or directed therapy of infected patients.
- 6) Loveena Oberoi, Nachatarjit Singh, Poonam Sharma, Aruna Aggarwal 2013, revealed a high prevalence of Beta lactamases in the ICU which emphasizes need for a continuous surveillance in the ICU's to detect resistant strains. 35.16% strains were ESBL producers, followed by 10.98% MBL and 5.4% AmpC producers. The co-production of ESBL/MBL/AmpC beta lactamases was observed in 19.04% strains.

- 7) Serine Altun, Zeliha Kocak Tufan, Server Yagci, Ufuk Onde, Cemal Bulut, Sami Kinikl and All Pekcan Demiroz 2013, concluded that AmpC, ESBL and MBL were the main resistance patterns of the strains evaluated in their study.

IV. Noteworthy Contributions In The Field Of Proposed Work:-

- 1) Vinita Rawat et.al, 2013 carried out a study of the detection of different beta lactamases and their co-existence by using various discs combination methods in clinical isolates of Enterobacteriaceae and Pseudomonas spp. and concluded that diagnostic problems posed by co-existence of different classes of beta lactamases in a single isolate could be solved by disc combination method by using simple panel of discs.
- 2) Silke polsfuss et. al,2011 studied on practical approach for reliable detection of AmpC beta lactamases producing Enterobacteriaceae and concluded that phenotypic methods for detection of AmpC are easy to perform and facilitate therapeutic decisions and epidemiological surveillance.
- 3) Saeide saeidi et. al, 2014 studied on the phenotypic and genotypic detection of extended spectrum beta lactamases (ESBL) producing E.coli from Urinary tract infections and concluded that TEM gene PCR is a rapid, sensitive and clinical useful test particularly for early detection of ESBLs , and monitoring of ESBLs production is recommended to avoid treatment failure and for suitable infection control.
- 4) Anand Manoharan et. al 2012 (ICMR ESBL study group) studied the phenotypic and molecular characterization of AmpC beta lactamases among E.coli, Klebsiella spp. And Enterobacter spp. from five Indian Medical Centres, and concluded that overall AmpC phenotypes were found in 12.5 %, multidrug resistance and ESBL co-carriage among them was high suggesting plasmid mediated spread which implicate rational antimicrobial therapy and continued surveillance of mechanisms of resistance among nosocomial pathogens.
- 5) Carlos M et.al, 2014 studied on Gram negative infections in Adult intensive care Units of Latin America and the Caribbean concluded that there are poor health outcomes due to MDR Gram negative bacteria infections and so urgent infection control strategies and local surveillance programs should be carried out.
- 6) Manu Chaudhary et. al, 2013 studied on Incidence, prevalence and control of MDR carbapenemase producing Acinetobacter baumannii in Indian ICUs and concluded that 81.71% of Gram negative organisms were carbapenemase producing. Vast collection of phenotypic data through microbial surveillance program enabled them to reach to the conclusion.
- 7) Mita D. Wadekar et.al, 2013 studied on the Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae and concluded that ESBL and MBL mediated resistance, which has created a therapeutic challenge for the clinicians and microbiologists, simple disk method can be routinely used to detect these common resistance mechanisms which will reduce the mortality and also the spread of such resistant strains.
- 8) Nirav P. Pandya et. al, 2011 studied on evaluation of various methods for the detection of Metallo beta lactamases (MBL) production in Gram negative bacteria and found that out of the total carbapenem resistant Gram negative bacteria 96.30% were MBL positive by Imipenem and Imipenem+EDTA combination disc method and the detection is of crucial importance.
- 9) Ravikant Porwal et.al, 2014 studied on Carbapenem resistant Gram negative bacteremia in an Indian ICU, and found that Carbapenem resistant bacteremia is a late onset infection in patients with antibiotic exposure in the ICU and carries a 30 days mortality of 60%; K. pneumoniae was the most common organism.
- 10) Afzal Azim et. al, 2010 studied on the Epidemiology of bacterial colonization at ICU admission with emphasis on ESBL and MBL producing GNB- an Indian experience, and concluded that 92% of patients were colonized with ESBL producing enterobacteriaceae on admission to ICUs, which were used as a guide for empiric antibiotic therapy targeted to the resistant bacterias.

V. Materials And Methods:

- 1) **Venue:** This prospective study will be carried out in the Department of Microbiology Bombay Hospital Ring Road Indore MP India.
 - 2) **Study Period:** The study will take a minimum of 2 years.
 - 3) **Strains for the study:** All the Gram negative bacterial strains isolated from the clinical specimens from the ICUs received in the department of Microbiology Bombay Hospital Indore within the stipulated study period will be collected and subjected to various phenotypic and genotypic methods for the screening and confirmation of the beta lactamases present in the isolated Gram negative strains.
- Inclusion criteria:-** All the samples from the ICU's showing Gram negative bacterial growth will be included in the study. Samples with Gram negative bacterial growth from all the ICUs i.e Medical and Surgical ICU, NeuroICU, Neonatal ICU, Isolation or Cubical ICU and Cardiac ICU will be included in the study.

Exclusion criteria:- The Gram positive bacteria, Fungal isolates, Mycobacteria isolated from the samples of the ICU's will not be included in the study. Also Gram negative bacteria isolated from specimens other than ICUs will not be included in the present study.

- 4) **Sample Size:** The following formula will be used for calculating sample size (Naing et al. 2006).

$$n = \frac{Z^2 P (1-P)}{d^2}$$

n = sample size

Z = Z statistic for a level of confidence of 95% Z value is 1.96.

P = expected prevalence of proportion

d = In proportion of one for 5% d is 0.05.

Based on the above formula the expected sample size is approximately 400 multidrug resistant gram negative bacteria isolated from clinical specimens of ICU's.

5) **Methodology:-**

All the Gram negative bacterial isolates from the clinical specimens of the ICU's, received in the department of Microbiology, Bombay Hospital Indore will be identified by manual methods using routine standard techniques. The Gram negative bacterial strains will be identified till species level as far as possible. Those Gram negative bacteria which are not identified by the routine manual methods will be identified by automated VITEK-2 compact method.

The Antibiotic Sensitivity Test will be performed on all the Gram negative bacterial strains by the standard Kirby Baurers disc diffusion methods as per the CLSI guidelines.

The history of the patients admitted to the ICUs and having Gram negative infections will be collected in the form of following protocol:-

- Age and Sex
- Type of Specimen sent for culture
- a. Type of ICU in which the patient is admitted
- b. Gram negative organism isolated
- c. The sensitivity pattern of the isolated Gram negative organism
- d. Brief history of the patient
- e. Antibiotic the patient was empirically receiving
- f. Phenotypic resistance mechanism of the isolated organism.

Phenotypic characterization of the drug resistance mechanism will be done as follows:-

Screening:-

- 1) **ESBL-** Decreased sensitivity to 2nd and 3rd generation cephalosporins will be considered as screen positive for ESBL.
- 2) **AmpC-** Resistance to Cefoxitin or Cefotetan will be considered as screen positive for AmpC.
- 3) **Carbapenemase -** Decreased sensitivity to Carbapenems will be considered as screen positive for carbapenemase production.

Confirmatory tests:-

- 1) **ESBL-** A difference in the zone size of 5mm between ceftazidime and ceftazidime+ clavulanic acid discs will be considered as confirmed ESBL producer phenotypically as per the CLSI guidelines.
Positive Control Strain:- Klebsiella pneumonia ATCC 700603 ESBL positive.
Negative Control Strain:- E.coli ATCC 25922.
- 2) **AmpC-** A difference in the zone size of 4mm between cefoxitin and cefoxitin + cloxacillin will be considered as confirmed AmpC producer (Serife altun et. al,2013, Vinita Rawat et.al, ,2013, Silke polsfuss et.al, 2011).
- 3) **Carbapenemase-** A positive modified hodge test (**MHT**) will be considered as carbapenemase producer as per the CLSI guidelines and a difference in the zone size of 7mm between Imipenem and Imipenem+ EDTA disc will be considered as MBL producer phenotypically.(Loveen Oberoi et al 2013, Vinita Rawat et al.,2013)
Positive MHT Control Strain:- Klebsiella pneumoniae ATCC BAA-1705.

Negative MHT Control Strain:- Klebsiella pneumoniae ATCC BAA-1706.

Genotypic confirmation and characterization of the beta lactamase genes:

The strains which are confirmed as ESBLs, AmpC, MBL and Carbapenemase producing phenotypically, will be confirmed genotypically for the production of the above mentioned genes by **Multiplex PCR**.

VI. Expected Outcome Of The Proposed Work:-

The present study will be helpful for:

- The epidemiological surveillance of the prevailing Gram negative bacteria in the ICUs
- Will determine the sensitivity pattern and the prevalent resistance mechanisms among the Gram negative bacteria in the ICUs.
- Will help in formulation of antibiotic policy and right choice of empirical therapy to reduce drug resistance.
- And ultimately to improve patient outcome.
- Will be helpful in detecting newer mechanisms of drug resistance prevailing among the multi drug resistant Gram negative bacteria.

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