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Research Paper

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KEYWORDS :

ISOLATION, IDENTIFICATION AND ANTIMICROBIAL SENSITIVITY PATTERN OF BACTERIAL ISOLATES FROM TRACHEAL ASPIRATE OF ICU PATIENTS AT TERTIARY CARE CENTRE IN SOLAPUR

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ABSTRACT

BACKGROUND:- *Critically ill patients acquire infection during their stay in intensive care unit (ICU) and frequency of these infections varies considerably in different populations and clinical settings1 .*

AIMS AND OBJECTIVES:-

1) The purpose of this study was to assess the prevalence of common pathogens in ICU.

2) To know the antibiotic susceptibility pattern of bacterial isolates from tracheal secretions admitted in ICU at tertiary care centre

.3) To determine the prevalence of extended spectrum B –lactamases (ESBLs) and Metallo -B-lactamases (MBL) was assessed .

MATERIAL AND METHODS:- *A total of 100 samples were studied over a one year period from March 2014 to March 2015. They were cultured and identified by standard biochemical tests . Antibiotic susceptibility test (AST) was performed according to CLSI guidelines. (CLSI 2014) Extended Spectrum B- Lactamase and Metallo-B-Lactamase was detected phenotypically .*

RESULTS:-Out of 100 samples studied the most common isolate was Acinetobacter baumanii ,followed by.Ecoli, Klebsiella.pneumoniae, Pseudomonas aeruginosa, Citrobacter.koseri, Staphylococcus aureus.

CONCLUSION:-*The findings of this study will help clinicians to formulate antibiotic policy for the treatment of the patients admitted in ICU. Producing a local antibiogram database will improve the knowledge of antimicrobial resistance patterns in so and will also help to improve treatment facilities*

INTRODUCTION:→

Critically ill patients acquire an infection during their stay in Intensive Care Unit (ICU) and the frequency of these infections varies considerably in different populations and clinical settings.[1- 3]

Critically ill Intensive care unit (ICU) patients are most vulnerable for developing these infections[4]. Compared with an average patient, an ICU patient has five to seven folds higher risk of nosocomial infection and ICU infections contributes to 20% to 25% of all nosocomial infections in a hospital [5] . LRTI is the most common infection in ICU specially in ventilated patients .Moreover, the ICU mortality of infected patients was more than twice that of non-infected patients.[4] Patients in the ICU have encountered an increasing emergence and spread of antibiotic resistant pathogens. Rates of nosocomial infections range from 5 to 30 % in ICU patients.[4] The increase risk of infection is associated with severity of patient's illness, length of exposure to invasive devices and procedures , increase patient contact with healthcare personal and length of stay in ICU.[4]

The tracheobronchial tree and oropharynx of patients on mechanical ventilation are frequently contaminated by microorganisms. The relation between this colonization and pulmonary infection, however, is not yet clear. Johanson et al. showed that 23% of patients colonized by bacteria later developed pulmonary infection.

As a result of these facts several studies have been published aimed at preventing, identifying and treating intra-hospital infections of the lower respiratory tract. One of the methods employed is sequential monitoring of the tracheal secretions of intubated patients in the ICU environment.[11-13]

Beta lactamases are the commonest cause of bacterial resistance to Beta lactum antimicrobial agents, which are used in treatment of various infections . With the increased use of antimicrobial agents bacteria responded to the variety of new B-lactamases including of extended spectrum B lactamase, plasmid mediated Amp C B lactamase and metallo -B- lactamases. Infection caused by multidrug resistant bacteria expressing B – lactamases pose serious challenges to clinicians because these bacteria are resistant to broad range of B- lactams including third generation cephalosporins and nosocomial infections caused by these organisms complicate therapy and limit treatment options [5]

Antibiotic overuse and misuse partly due to incorrect diagnosis; as well as irrational and counterfeit antibiotic market combinations; and irregular consumption due to either wrong prescription or poor compliance; all contributes to the wide spread drug resistance among the hospital acquired organisms.[6-7]

The patterns of organisms causing infections and their antibiotic resistance pattern vary widely from one country to another; as well as from one hospital to other and even among ICUs within one hospital[7]

Therefore the characterisation of antibiotic susceptibility pattern of B- lactamase producing microorganisms can lead to successful infection control involving antimicrobial stewardship and public health interventions aimed at controlling the emergence of such life threatening multidrug resistant bacteria[8]

Hence this study was undertaken to detect bacterial pathogens and determine the antimicrobial resistant pattern of clinically relevant bacteria producing extended spectrum B- lactamase

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(ESBL) and metallo- B- lactamase (MBL) from tracheal aspirates of patients admitted in ICU.

Knowledge of sensitivity pattern of the organism isolated in ICU is helpful in selecting empirical therapy. Therefore the purpose of the study was to examine the antimicrobial susceptibility pattern of gram negative isolates from tracheal aspirate admitted in ICU at tertiary care centre in Solapur over a one year period from March 2014 to March 2015.

AIMS AND OBJECTIVES:→

1) The purpose of this study was to assess the prevalence of most common pathogens in ICU.
2) To know the antibiotic susceptibility pattern of bacterial isolates from tracheal secretions admitted in ICU at tertiary care centre
3) To determine the prevalence of extended spectrum B –lactamases (ESBLs) and Metallo-B-lactamases (MBL) was assessed .

Study setting :-> This study was conducted in Department of Microbiology at tertiary care centre

Study Period: -- 100 Samples of the patients admitted in the ICU from a period of March 2014 to March 2015 were included in the study.

Study sample:→

MATERIALS AND METHODS: →

100 Tracheal aspirates were collected from patients admitted in ICU in tertiary care centre .. The Centre for Disease Control and Prevention (CDC) defines ICU associated infections as those that occur after 48 hours of ICU admission or within 48 hours after transfer from an ICU.[19]The following data were collected from the patients enrolled in the study :-->name ,age, gender ,underlying illness, date of admission ,date of endotracheal insertion ,date of endotracheal aspiration collection, duration of hospitalization, duration of mechanical ventilation, details of prior antibiotic therapy, clinical diagnosis, X ray report. Other relevant data were collected from microbiological studies from laboratory. Endotracheal aspirate (ETA) sample was collected after hand washing in an aseptic manner. ETA was collected from suction catheter. It was gently introduced through endotracheal tube for a distance of approx. 25-26 cm. After collection of endotracheal aspirates from patients in a sterile tube sample were immediately transferred to microbiology laboratory for gram staining and culture. Samples were mechanically liquefied and homogenised and then gram stain and culture was performed. The smears were gram stained and examined. The smears were examined under low power field (HPF) and under oil immersion for examination of squamous epithelial cells and pus cells . .Samples were inoculated on MacConkey agar, Blood agar. Samples were incubated at 37˚c for 24 hours. . The presence of significant colony count of 10^5 was counted[8,14]. The Gram positive isolates were subjected to catalase test, coagulase test, mannitol production, bile esculin etc. The Gram negative were subjected to Indole test , Methyl red test , citrate utilisation, hydrolysis of urea, Triple sugar iron test, decarboxylase test, Oxidative Fermentative test etc and were furthered identified on the basis of standard microbiological techniques. Antibiotic sensitivity test was performed using Kirby –Bauer disk diffusion method using standard disk supplied by Hi media laboratories ,Mumbai and antibiotic susceptibility results were interpreted according to CLSI guidelines.(CLSI 2014) Multidrug resistance was defined as resistance to three or more of antimicrobial agents belonging to different structural classes.

INCLUSION CRITERION :→ 1)All the patients who were >or=18 years and 2) Those who were on mechanical ventilation for > or= 48 hours.

EXCLUSION CRITERION:→

1) All patients who have received antimicrobial therapy

PHENOTYPIC TESTS FOR ESBL PRODUCTION:→

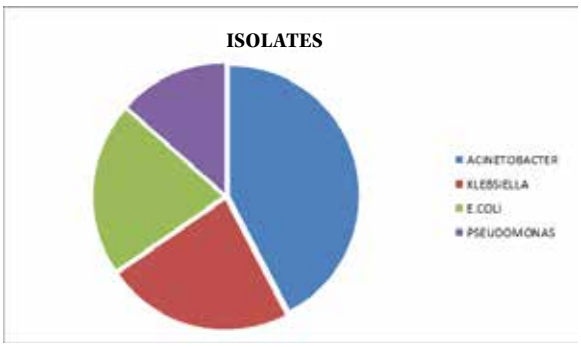
Isolates were first screened for ESBL production by the disk diffusion method (screening test) using cefotaxime ,ceftriaxone,,ceftazidime and later on confirmed by cephalosporin/clavulanate combination disk(disk potentiation test) and double disk synergy test. *E.coli I* ATCC 25922 (non ESBL producer) was used as control strain.[17]

PHENOTYPIC METHODS FOR MBL DETECTION:→

The isolates were tested for sensitivity to Imepenem (10 µg) using Kirby – Bauer method as recommended by CLSI (2014). All the isolates with zone of inhibition less than 16 or which demonstrated heaping or if zone was more than 16 but less than 20 .these isolates were confirmed by Modified Hodge test and Impenem –EDTA double disk synergy test.[17]

RESULTS :-

Out of 100 samples collected 57 were males and 43 were females.]



ORGANISM	TOTAL ISOLATES	% OF ISOLATES
ACINETOBACTER	32	46.06%
KLEBSIELLA PNEUMONIAE	20	35%
E.COLI	14	22.2%
PSEUDOMONAS AERUGINOSA	8	14.7%
NON FERMENTOR GNB	4	8.0%
CITROBACTER	4	8.0%
ENTEROBACTER AEROGENS	4	8.0%
STAPHYLOCOCCUS AUREUS	8	14.7%
ENTEROCOCCUS	3	6.32%
PROTEUS	3	6.32%

ORGANISM	ESBL PRODUCER	MBL PRODUCER
ACINETOBACTER	29	4
KLEBSIELLA	9	--
E.COLI	10	--
PSEUDOMONAS	7	--
NON FERMENTER GNB	3	--
CITROBACTER	3	--

Table 2: Antibiotic resistance rates (%) for predominant Gram-negative bacilli recovered from trachi aspirate of ICU patients.

Antibiotics	Acinetobacter spp. (n=32)	K. pneumoniae (n=20)	Pseudomonas spp. (n=8)	E.coli (n=
Amikacin	17.2	14.8	10.5	8.3
Ampicillin	NT	100	NT	100
Cefotaxime	17.3	15.6	14.8	13.3
Cefoxitin	40.5	35.5	22.2	16.7
Ciprofloxacin	20	14.3	13.4	12.7
Cotrimoxazole	86.4	85.7	86.5	83.3
Erythromycin	NT	NT	NT	25
Gentamicin	23.6	19	17	15
Imipenem/meropenum	92.6	66.5	76.2	55
Gentamycin	80	64.3	51.4	66.7
Ceftazidime	19.1	17.1	9.7	7
Ceftriaxone	19	10	9	4

Staphylococcus aureus (10.4%), other Gram negative bacteria (5.9%) and Viridans Streptococci (2.9%). *Pseudomonas aeruginosa* were most sensitive to amikacin (81.4%) and ciprofloxacin (70.3%). All Pseudomonal isolates were resistant to cefotaxime. Enteric Gram Negative bacteria were most sensitive to amikacin and chloramphenicol (74.0%) and all were resistant to ampicillin and cephalexin. All the gram positive bacteria isolated were sensitive to vancomycin. Among all the isolates, 88.8% of *Pseudomonas aeruginosa*, 66.6% of enteric gram negative bacteria and 55.5% of Gram positive bacteria were multidrug resistant.

Our result revealed that *Acinetobacter , Klebsiella ,E.coli, Pseudomonas, Citrobacte*r were prominent isolates. High degree of antibiotic resistance were seen among *Acinetobacter, Klebsiella ,E.coli, Pseudomonas* specially to cephalosporins, fluroquinolones and aminoglycosides.

Our study showed that *Acinetobacte*r spp is the organism that is frequently isolated from ICU. It is most commonly isolated from tracheal aspirates. It is highly resistant to ceftriaxone , ceftazidime moderately resistant to fluroquinolones and sensitive to meropenum / imepenum. Some studies however have found good sensitivity of *Acinetobacter.baumanii*. *Acinetobacter* spp is an emerging infection in an ICU setting.

CONCLUSION:→

We conclude that *Acinetobacter baumanii* is the most common etiological agent in ICU .There is an alarming increasing rate of resistance to cephalosporins , B-lactamases inhibitors and carbapenems against predominant organisms .Although meropenem is still sensitive to most pathogens but resistance is rising. Respiratory infections and antimicrobial resistance in the ICUs is a major deterrent to patient outcome, increasing duration of patient stay as well as expense. Reduction of the same is both challenge and goal of all intensive care units around world. Strict infection control measures like universal precautions and stringent adherence to hand washing practices[7,13];formulation and antibiotic policy; Survillence activities[3,7]; appointment of infection control practitioners; might be required for the same for which further research is advocated.

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