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ASSESSMENT OF ANTIBIOTIC SUSCECPTIBILITY PATTERN OF GRAM NEGATIVE PATHOGENS ISOLATED FROM INTENSIVE CARE UNIT PATIENT OF "COPD" IN SOUNTH ZONE OF TAMILNADU, INDIA.

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ABSTRACT

Extended spectrum of Beta-lactamase (ESBL) and emergence of antibacterial resistance among nosocomial pathogens in the intensive care unit of hospitals created serious health care concern. The resistance developed by G-ve bacteria against Beta-lactam antibiotics. The aim of the study to evaluate the prevalence of multidrug resistance organism and incidence of ESBL producers in G-ve bacteria isolated from endotreacheal secretion of COPD patient of private hospital, Tamilnadu. 289 tracheal secretion samples collected and screened for the presence of bacterial pathogens. The pathogens were identified using selective media technique and confirmed with Hi-chrome agar and automatic technique biomerius. The ESBL producer's screened by double disc synergy test. Among 289 samples, 232 showed culture positive and 57 samples showed culture negative. In culture positive, 182 G-ve bacteria and 27 G+ve bacteria, 23 fungus were isolated. In 182 G-ve bacteria, Klebsiella sps (24%) as the predominant pathogens followed by Pseudomonas sps(20%), Enterobacter (10%), Acinetobacter sps (3%), E.coli (6%). Of the identified pathgoens, 50% were found to be ESBL producers and 31 % were MDR, 10% Acinetobacter and 9% E.coli. Colistin was highly susceptible (60-80%), followed by Imipenem and Meropenen (50-60%), Cefaperazone/Sulbactum (10-15%) and Piperacillin/Tazobactum (5-40%) against Gram negative bacteria. From the above results it can be conclude that Klebsiella sps and Pseudomonas sp was the most common pathoges associated with COPD patient of intensive care unit hospitalized elder in private hospital,

Colistin and Imipenem is an drug of choice to treat the infection caused by carbapnemase producing multi-drug resistant gram negative bacteria.

KEYWORDS: COPD, Gram negative bacteria, ESBL.

INTRODUCTION

Beta –lactamases are bacterial enzymes, ESBLs are Beta-lactamases capable of conferring bacterial resistance to the penicillins, first, second and third generation cephalosporins, aztreonam by hydrolysis of these antibiotics and are inhibited by Beta lactamase inhibitors such as Clavulanic acid. First described in Germany (1983), France (1985) among Klebsiella spp., ESBLs exits in every region of the world and in most genera of enterobacteria. Currently, ESBLs are becoming a major threat for patients in the hospital, long-term care facilities and community. These bacteria have not only caused outbreak but have become endemic in many hospitals throughout the world.[1] When a significant proportion of Gram negative isolates in a particular units is ESBL producers, empirical therapy may change towards use of Imipenem, quinolones, Beta lactam/ Beta –lactamase inhibitor combinations. In some centers this has been associated with emergence of imipenem resistance in Pseudomonas aeruginosa, Acinetobacter baumanii and in ESBL-producing organisms themselves.[2,3]

Clinical and Laboratory Standard Institute's (CLSI) recommendations[4] that clinical microbiology laboratories perform specialized tests for detection of ESBL, many clinical microbiology 297-298 Indian J Med Res, April 2008 laboratories make no effort to detect ESBL production or are ineffective at doing so.[5] In a 1998 survey of 369 American clinical microbiology laboratories, only 32 % (117 of 369) reported performing tests to detect ESBL-producing Klebsiella were reported by their original clinical laboratories as cefotaxime resistant. Situation of both ESBL prevalence and their detection is definitely worse in resource-poor settings of developing nations. Factors like overcrowding, poor nutritional and hygiene status and lack of infection control measures combined with antibiotics misuse has led to high endemic levels of these resistant bugs. In some centers from India as many as 86 % of Klebsiellae have been found to be ESBL producers.[6]

Bacteria are isolated from sputum in 40 to 60% of acute exacerbation of COPD. Three predominant bacterial species isolated from patients experiencing exacerbation of COPD are nontypable Haemophilus influenza, Morexella catarhalis, and Streptococcus pneumonia.[14]

Several recent studies have suggested that Gram negative species, more commonly associated with bronchiectasis patient, are also isolated in COPD, and that there is a correlation between lung function and the bacterial species isolated. Haemophilus influenza is still the most common isolate overall in these studies. In patient with airflow obstruction, Streptococcus pneumonia and other Gram positive cocci are more frequently isolated, whereas Pseudomonas aeruginosa and other Gram negative bacilli account for a significant number of isolates in patient with severe airflow obstruction, but are rare in mild case.[15]

Monso et al studied 40 COPD patient during a stable phase of their illness and found positive during PSB culture defined as $>10^3$ colony forming units (cfs) mL^{-1} in one-quarter of patient. Haemophilus influenza and Streptococcus pneumonia were the predominant species detected. Most bacterial species that infect the bronchial tree also form part of the commensal flora of the nasopharynx Haemophilus influenza and opportunistic pathogens e.g Pseudomonas aeroginosa.[16]

Sample collection

289 Tracheal secretion samples were collected in sterile container from hospitalized COPD patient. The present study was conducted at the o of private hospital during the period from November 2012 to October 2013 in the south Tamilnadu, India. Patient who had dyspenea, low grade and intermittent fever, chills, chronic cough with sputum production, cold, breathlessness, purulent sputum yellow with blood stained, old TB, systemic hypertension, Diabetic melintensis and risk factors, such as alcohol consumption, tobacco use, occupational exposure to smoke and fumes, dust and use of bronchodilator.

Patient were clinically stable. The processing of the specimen were done as per (CLSI) guideline. The following medium used for the isolation of organism, EMB agar, Blood agar, MacConkey agar.

Identification of organism

The following medium used for the isolation of organism, EMB agar, Blood agar, MacConkey agar.

Growth on media MacConkey used for isolation and cultivation of lactose fermenting and non-lactose fermenting bacteria. Blood agar also used to cultivation of Gram negative bacteria and used to isolate Staphylococcus aureus and Streptococcus species, Candida species. Chocolate agar used to isolate Haemophilus influenae.

Biochemical tests

Catalase tests: A colony of tested bacteria was mixed with 3% H_2O_2 on the slide and positive results were indicated by air bubbles formation.[7]

Coagulase test: 0.5 ml of citrated rabbit plasma (diluted 1 in 5 with saline) mixed with 5 drops (250 µl) of overnight broth culture or small amount of the colony growth of Staphylococcus aureus and Incubated at 37^0 C for 4 hours. A tube of plasma mixed with sterile broth was included as a control. Formation of clots in 1-4 hrs indicates a positive test. If no clot is observed at that time, reincubate the tube at room temperature and read again after 18 hours.[8]

Oxidase: Filter paper moistened with freshly prepared 1% oxidase reagent, a colony of tested of bacteria smeared on the filter paper, and positive results were indicated by development of deep blue color at the site of colonies smeared within 10 seconds. Negative result the colour of the colonies remain unchanged.[9]

Imvic

Indole: Tryptophan broth inoculated with the tested bacteria and incubated at37^0 C for 24 to 48 hrs. 0.2 ml of Kovac's reagent added to the 0.5 ml culture broth. Formation of red-violet ring within minutes indicates a positive test. Development of yellow ring indicate negative test.[10]

MR and VP test: A colony of tested bacteria inoculate in MR-VP medium and incubated at37^0 C for 24 to 48 hrs. 0.04 % methyl red added to the broth of culture. Development of stable red color indicate MR positive. Negative no color change. 5% naphthol and 40 % KOH (VP reagent added to the VP medium. Development of pink colour within 2-5 minutes indicate positive test. No pink color within 15 minutes indicate negative test. The test should not read after for over 1 hour it leads false positive interpretation.

Citrate utilization

A colony of tested bacteria inoculate in simmon' citrate medium and incubated at37^0 C for 24 to 48 hrs. Development of deep blue colour with in 24 to 48 hours of incubation indicate positive test. Negative test indicated by no colour change of the citrate medium.

Tsi

A colony of tested bacteria inoculate in TSI (Triple sugar iron agar) medium and incubated at37^0 C for 18 to 24 hrs. Observe the color change in the slant and butt and also observed the development of black precipitation indicate H_2S production. Alkaline slant and alkaline butt (K/K) indicate no carbohydrate fermentation and non-fermentation. Alkaline slant and acidic butt (K/A) indicate glucose fermented and lactose, sucrose was not fermented it showed the tested bacteria were non-lactose fermented. Acidic slant /acidic butt (A/A) all sugars (lactose, glucose and sucrose) were fermented, it indicate lactose fermenting bacteria.[11]

Antimicrobial sensitivity test

All isolated bacteria were subjected to antimicrobial susceptibility test by Kirby-Bauer disc diffusion method. The antimicrobial used were amikacin (30 µg/disc), amoxicillin/clavulanic acid (30 µg/disc), Cefdinir (5 µg/disc), Cefepime (30 µg/disc), cefixime (5µg/disc),, Cefaperazone (75 µg/disc), Ceftazidime (30 µg/disc), Ceftriaxone(30 µg/disc), Cefuroxime(30 µg/disc), Ciprofloxacin (5 µg/disc), Gentamycin (10 µg/disc), Levofloxacin (5 µg/disc), Piperacillin(100 µg/disc),, Azithomycin(15 µg/disc), Cephoxitin (30 µg/disc), Linezolid (30 µg/disc), oxacillin(1µg/disc), Vancomycin (30 µg/disc).[12]

ESBL- detection

Double disk synergy test was performed using disks of Ceftazidime (30 µg/disc), Ceftriaxone(30 µg/disc), Cefotaxime (30 µg/disc), amoxicillin/clavulanic acid (30 µg/disc) and combined drug of cefotaxime /clavulanic acid (30/10 µg/disc), cefotazidime /clavulanic acid (30/10 µg/disc). The disks were placed at a distance of 30 and 16 mm from each other and incubated for 37^0 C for 24 hrs. Increased zone of inhibition in the combined disks (cefotaxime /clavulanic acid (30/10 µg/disc), cefotazidime /clavulanic acid (30/10 µg/disc) than the Cefotaxime (30 µg/disc), amoxicillin/clavulanic acid (30 µg/disc). This type of sensitivity pattern producing bacteria considered harboring ESBL. (Ananthakrishnnan, et al., 2000).[13]

Quality control

According to the CLSI guidelines, quality of used media, reagent, antibiotics, checked with quality control strains of ATCC Pseudomonas aeruginosa 27853, ATCC E.coli 25922, ATCC Staphylococcus aureus 25923.

RESULT

Endo tracheal samples (n=28) were collected ffrom ICU of private hospitals, south India and processed for the isolation of pathogenic bacteria. Bacteria were isolated according to Bergey's manual. 232 samples out of 289 samples tested showed the presence of the microbial infection, where as the remaining 57 samples culture negative (Table 1) (Fig 1). Among culture positive, 182 G-ve bacteria and 27 G+Ve bacteria, 23 Candida sps were isolated. Based on the colony morphology and biochemical characterization of the bacteria isolated from the culture positive sample demonstrated 4 to 5 type of gram negative bacteria include Klebsiella sps, Pseudomonas sps, Enterobacter, E.coli, Acinetobacter. However the Gram positive pathogens detected in the samples not included in the study.

Among 182 G-ve bacterial infection, Klebsiell sps (68) (24%) as the predominant pathogens followed by Pseudomonas sps (57) (20%) and Enterobacter (29) (10%), Acinetobacter sps (10) (3%) and E.coli (18) (6%). (Table. 2) (Fig. 2,3). Of the identified pathogens, 50% were found to be ESBL producers and 31 % were MDR, 10% Acinetobacter sps, 9% E.coli. Colistin was highly susceptible (60-80%) followed by Imipenem , Meropenem(50-60 %), Cefaperazone/ Sulbactum (10-15%) and Piperacillin/Tazobactum (5-40 %) (Table. 4) (Fig 4) against Gram negative bacteria.

From above results it can be concluded that Klebsiella sps and Pseudomonas sps was the most common pathogen associated with COPD patient of intensive care unit hospitalized elder in private and Colistin and Imipenem is an drug of choice to treat the infection caused by carbapenemase producing multi-drug resistant gram negative bacteria.

Table 1: Culture positive and Negative details from the Clinical specimen

TOTAL NO OF SAMPLE TESTED	289
CULTURE POSITVE	232
CULTURE NEGATIVE	57
NO OF GRAM NEGATIVE	182
NO OF GRAM POSITIVE	27
FUNGUS	23

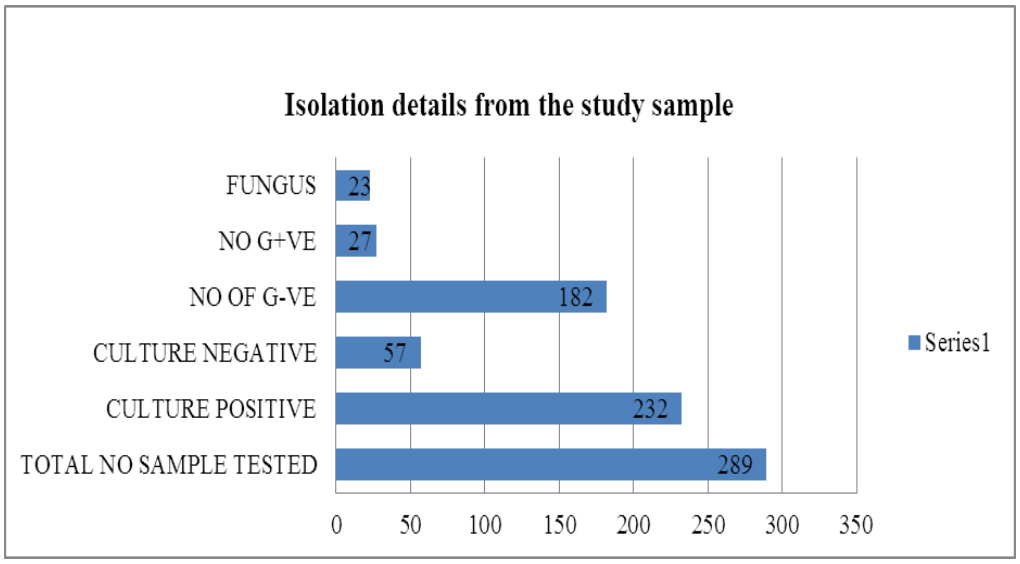


Fig 1: Isolation details from the study sample

Table 2: Prevalence of pathogens in the samples

ORGANISM ISOLATED	No of isolates	%
KLEBISELLA	68	24
PSEUDOMONAS AERUGINOSA	57	20
ENTEROBACTER	29	10
ACINETOBACTER	10	3
E.COLU	18	6
MRSA	4	1
STAPHYLOCOCCUS AUREUS	20	7
STREPTOCOCCI PNEUMONIAE	3	1
NORMAL FLORA	57	20

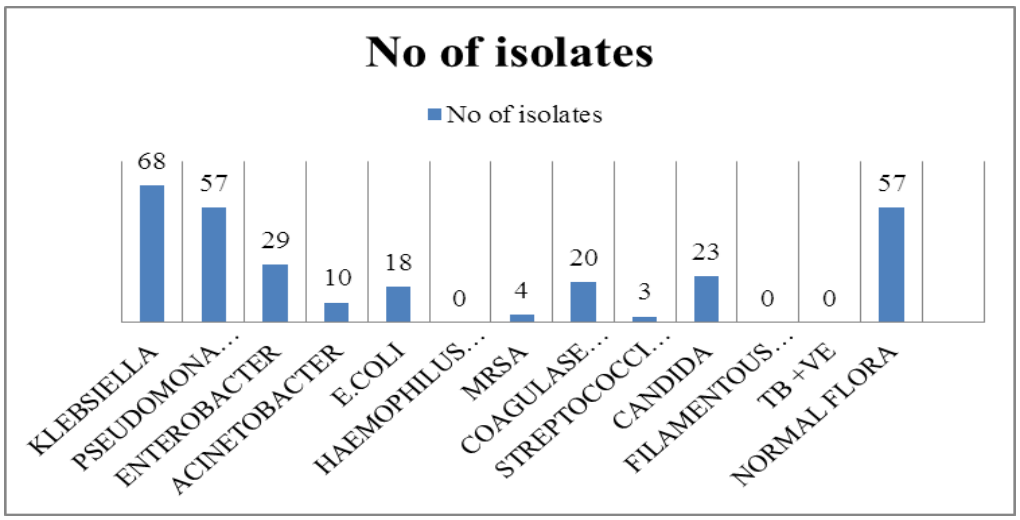


Fig 2: Graphical representation of microbial details isolated from the ICU –COPD patient

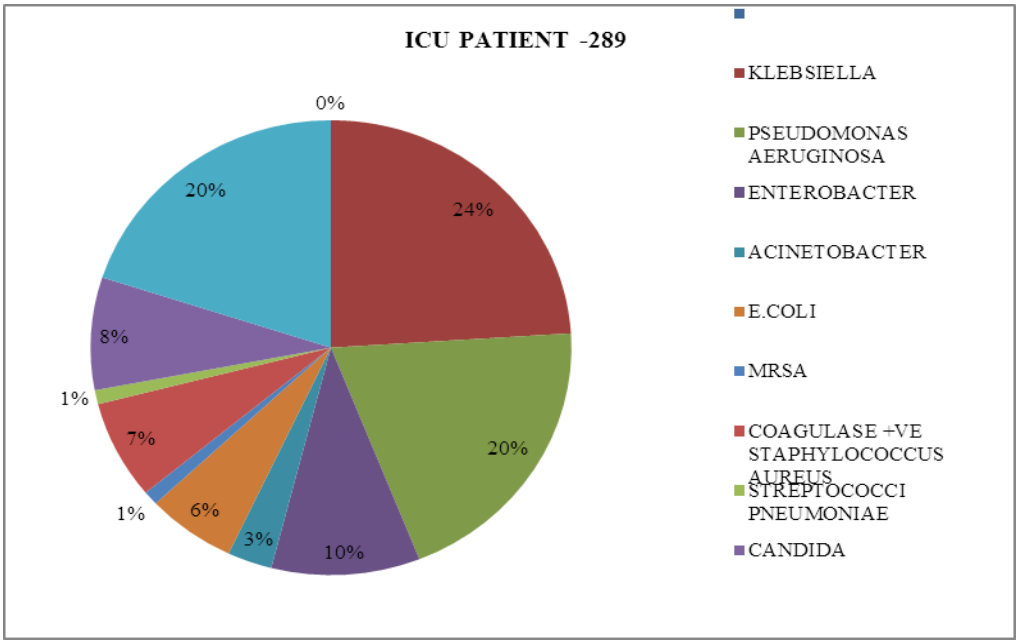


Fig 3: Diagrammatic representation of microbial details isolated from the ICU COPD patient

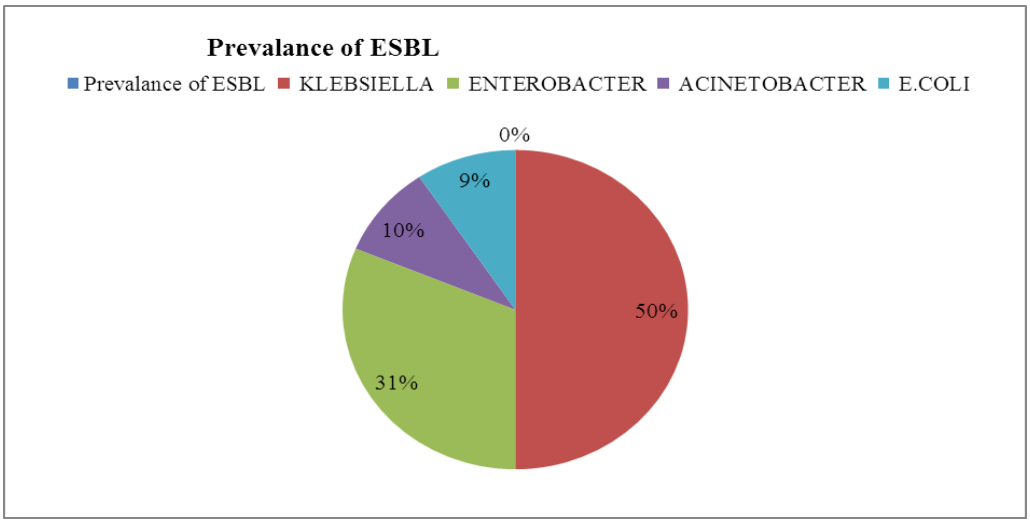
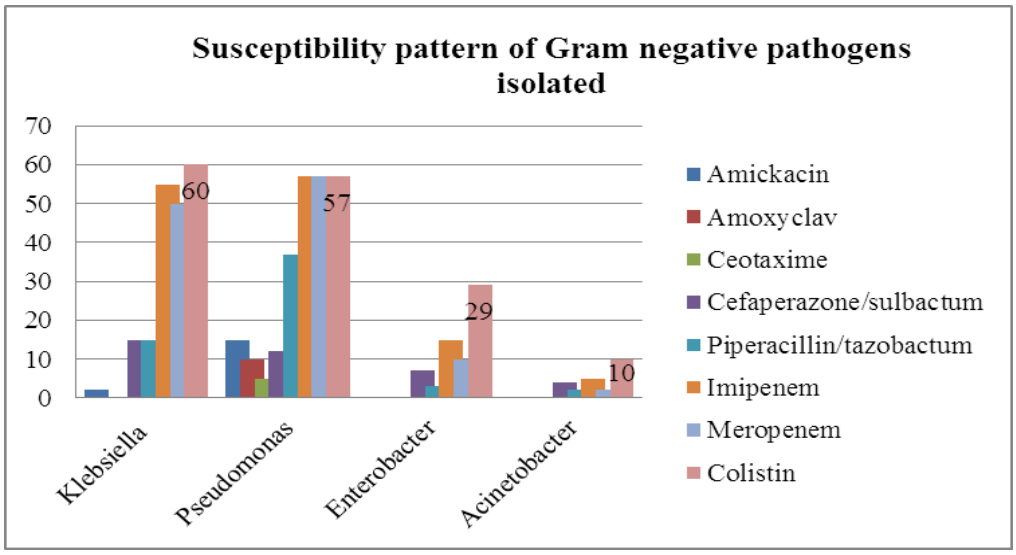


Fig 4. Prevalence of ESBL

Table 3: Susceptibility pattern of Gram negative pathogens isolated

Antibiotics	Klebsiella	Pseudomonas	Enterobacter	Acinetobacter
Amickacin	2	15	0	0
Amoxyclav	0	10	0	0
Ceotaxime	0	5	0	0
Cefaperazone/sulbactum	15	12	7	4
Piperacillin/tazobactum	15	37	3	2
Imipenem	55	57	15	5
Meropenem	50	57	10	2
Colistin	60	57	29	10



CONCLUSION

It is concluded from the study resulted in prevalence of resistance pattern of different pathogens from south zone part of India and is found to be significant high for antibiotics Imipenem, Piperacillin/Tazobactum, Cefaperazone/Sulbactum and Meropenem. Infection with Acinetobacter in COPD patient are one of the major concerns to treat because of their intrinsic and acquired resistance.

Colistin displayed significant susceptibility against the ESBL and MDR. This study very usefulness for the clinicians. Further this study showed Klebsiella sps and Pseudomonas sps is most predominant bacterial pathogen from the hospitalized COPD patient of intensive care unit.

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