IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 12 Ver. IV (Dec. 2017), PP 87-90
www.iosrjournals.org

Bacterial Isolation And Antibiogram with Esbl Production of Klebiella Species From Exudates From Govt General Hospital ,Guntur.

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Abstract
Introduction*: The number of organisms developing resistance to commonly used antibiotics is increasing among the various generations.Klebsiella species is associated with different types of infections and there is a preponderence of multidrug resistance and ESBL production.*
Materials and Methods *: A total number of 2,240 exudate samples collected from March 2017 to October 2017 from Govt General hospital,Guntur were included in the study . Bacterial isolates were identified by their standard Microbiologicaltechniques and were subjected to antibiotic sensitivity testing using modified Kirby-Bauer disc diffusion method.*
RESULTS *: Of 2,240 exudate samples processed ,746 (33.30%) samples were culture positive for various organisms.Itconisisted of 599 (80%) Gram negative bacteria. Of 746 culture ,368 (50%) were positive for Klebiella species,87 (11.76%) were Positive for Pseudomonas aueriginosa ,85(11.40%) were Escherichia coli, 51 (6.83 %) were Proteus species , 08 (2.17%) were Citrobacter and 06(1.6%) were acinetobacter . 95 (25.8%) were Staphylococcus aureus ,27(7.33%) were CONS and 19(5.16%) were Streptococci .The frequency of ESBL producers in our study was 72.5% of all Klebsiella isolates.*
Conclusion *: Klebsiella species turns out to be the most frequently isolated organism with high ESBL prodution. Since the exact national scenario of antimicrobial resistance is not known in India owing to the absence of a central monitoring agency, we can asses the possible factors that can favour an antibiotic policy for the proper and effective use of antibiotics.*

Date of Submission: 27-11-2017 Date of acceptance: 09-12-2017

I. Introduction

Controlling infections is going to be a tough job in developing countries like India where infectious diseases still hold high Mortality and Morbidity. The National scenario of antimicrobial resistance is not known in India due to the absence of central monitoring agency. In view of this fact and due to the geographical and time based variations in antibiotic resistance and sensitivity that have been reported by many studies this study was undertaken with the following objectives at Guntur Government General Hospital,AP,

1. To identify the group of most common pathogens
2. To identify the class of drugs against which resistance has emerged
3. To assess the possible factors that can favor the development of antibiotic policy for proper and effective use of antibiotics.
4. To identify the resistance pattern of Klebsiella species and their ESBL production.
5. Inappropriate and irrational uses of antibiotics in humans and animals for therapeutic and non-therapeutic use (as growth promoters) have been focused as main causes for the emergence of hospital and community acquired resistant infections by World Health Organization (WHO).

Klebsiella pneumoniae (Klebsiella) are ubiquitously present and reported worldwide. In recent years, Klebsiella have become important pathogens in nosocomial infections [1], which have been well documented in United States [2] and India [3]. Epidemic and endemic nosocomial infections caused by Klebsiella species are leading causes of morbidity and mortality [4].Klebsiella pneumoniae is a Gram-negative,non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin and intestines [5]. Klebsiella is most frequently recovered from clinical specimens and can cause a classic form of primary pneumonia and a variety of extrapulmonary infections, including enteritis and meningitis in infants, urinary tract infections in children and adults and septicaemia[6]. Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of Klebsiella and the development

of multidrug-resistant strains that produce extended–spectrum betalactamases (ESBLs) [7]. In recent years, there has been an increase in the incidence and prevalence of ESBLs [8].

II. Material And Methods

This study was carried from March, 2017 to October , 2017 at the Department of Microbiology ,Government General Hospital ,Guntur. A total of 2,240 exudate samples which consisted of CSF ,pus ,sputum ,and all sterile body fluids were collected with universal safety precautions and were transported to the laboratory at Guntur Medical Collegewithout delay. The pus samples were either aspirated by disposable syringes or collected onto sterile cotton tipped swabs. Sputum and other sterile fluids were collected into an autoclaved sterile screw top containers [12]. Samples were inoculated on Blood agar and Mac Conkey agar and incubated overnight at 37ºC.All the isolates were identified using standards as per the clinical and laboratory standards institute guidelines.Klebsiella pneumoniae strains were identified by their morphology and biochemical characteristics. Morphology of Klebsiella pneumoniae identified were large, dome-shaped, mucoid colonies on blood agar and lactose fermenting colonies on Mac Conkey agar. In Gram-staining, Gram-negative, short, plump, straight rods were seen. The biochemical characters identified were negative indole test, negative methyl red test, positive Voges-Proskauer test, positive citrate utilization test, positive urease test , triple sugar iron (TSI) ,acid and abundant gas production from glucose, lactose, sucrose, maltose and mannitol sugar fermentation tests .[5]Escherichia coli strains were identified by their morphology and biochemical characteristics. Morphology was identified by smooth colonies on blood agar and lactose fermenting colonies on Mac Conkey agar. In Gram-staining, Gram-negative, straight rods were seen. The biochemical characters identified were positive indole test, positive methyl red test, negative Voges-Proskauer test, negative citrate utilization test, acid/acid with gas reaction triple sugar iron (TSI) ,acid and gas production from glucose, lactose, sucrose, maltose and mannitol sugar fermentation tests .[5]

All other microbes were identified using as per the clinical and laboratory standards institute guidelines.Antimicrobial susceptibility testing: Antibiotic sensitivity of clinical Klebsiella pneumoniae isolates was done by Bauer's and Kirby's disc diffusion method according to the CLSI guidelines 2015 (CLSI, 2015)[13]. Commercially available antibiotics, carbapenems -10mcg, amikacin - 30mcg, piperacillin/tazobactum-100+10 mcg , ciprofloxacin 5 mcg ,cotrimoxazole 30mcg, gentamicin - 10mcg, ampicillin+sulbactum - 10mcg/10mcg, cefaperazone/sulbactum75=30mcg cefotaxime - 30mcg, ceftriaxone - 30mcg, were used.NCCLS screening test Isolates showing an inhibition zone size of ≤25 mmwith cefriaxone (30 μg) and ≤27 mm with cefotaxime (30 μg) were identified as potential ESBL producers. (NCCLS Screening test)[14].

III. Results And Discussion

A total of 2,240 sputum, Pus and sterile body fluids sent to the department of Microbiology were processed . 746 [33.3%] bacterial isolates were culture positive (Table-1). Of them 368 (49.32%) were Klebsiellaspecies ,87 [11.7%] were pseudomonas species, 85 [11.40%]were Escherichia coli ,51 [6.83%] were Proteus species ,8 [2%]were Citobacter and 06[1.6%] were Acinetobacter(Table -2). This constituted the growth of Gram negative bacteria which was 599 [80%] of the positive cultures.Gram positive organisms isolated were 147 [20%]. They constituted 95 [25.8%] of staphylococcus aureus ,27 [7.33%] were coagulase negative staphylococci ,19[5.16%] Streptococci .

The sensitivity and resistance pattern of the Klebsiella isolates to various antibiotics in our study is shown in the Table-5. Of the 368Klebsiella isolated, 257(70 %) were ESBL producers. Klebsiella species were highly sensitive to carbapenems [94%] ,Amikacin [76%] , Gentamicin [73%] Piperacillin tazobactum combination [70%] ,Cefperazonesulbactum combinations [60%]. They were also sensitive to ciprofloxacin [53%]Cotimoxazol [45%].They were Risk factors associated with ESBL production include – prolonged hospital or intensive care stay, use of multiple courses of antimicrobial therapy, particularly extended spectrum cephalosporins. Hence, indiscriminate use of cephalosporins and broad spectrum antibiotics should be avoided.

IV. Conclusion

The present study reveals 80% of isolation rate of Gram negative bacteria from various clinical samples with highest sensitivity to carbapenems and Amikacin and highest resistance to cefotaxime and ceftriaxone. The rarer organisms like Pseudomonas[11.70%] and Proteus[6.83%] have increased isolation.staphylococcus aureus isolation is high[25.8%] of total isolates.These isolates were sensitive to higher antibiotics like Carbapenems [94%], Amikacin[76%],Piperacillin tazobactum combinations [70%]. Basic drug like Gentamicin showed higher sensitivity [73%]. Restricted use of these drugs because of cost and the need for parenteral assistance may be the contributing factor for remaining sensitive still.This supports the possibility of changing pattern of sensitivity with time difference.

Among antimicrobials used in our study for culture sensitivity highest resistancewas showed by ampicillin/sulbactum,Cotimoxazole and Ciprofloxacin.,which might be due to the easy availability over the counter and easy self administration.

ESBL isolates of Enterobacteriaceae family is major problem worldwide. Indiscriminate use of third generation Cephalosporins to treat Gram negative bacterial infection is partly responsible for the emergence of resistance to beta – lactam antibiotics. Strict adherence to the hospital antibiotic policy and good infection control practices can play a significant role in reducing the emerging drug resistance.However only screening tests were performed for detection of ESBLs in our study, confirmatory studies are required for further evaluation.

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Table 1

Gram negative isolates	Gram negative isolate %	Gram positive isolates	Gram positive isolate %
599	80%	147	20%

Table 2

Total no of samples	No of positive isolates	% of positivity
2240	746	33.3%

Table 3 Gram negative isolates

s.no	Isolate	No	Percentage
1	Klebsiella	368	49.32%
2	Pseudomonas	87	11.70
3	Escherichia coli	85	11.40
4	Proteus	51	6.83%
5	Citrobacter	08	2.17%
6	Acinetobacter	06	1.6%

Table 5: sensitivity pattern

s.no	Drug	Sensitivity
1	Carbapenems	94%
2	Amikacin	76%
3	Gentamycin	73%
4	Piperacillin tazobactum	70%
5	Cefperazonesulbactum	60%
6	Ciprofloxacin	53%
7	Cotrimoxazole	45%
8	Ampicillin sulbactum	20%
9	cefotaxime	30%
10	Ceftriaxone	30%

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