Research Paper Microbiology

Prevalence of Multidrug Resistance (MDR) Klebsiella Species in Ventilated Associated Pneumonia (VAP), Urine Tract Infection (UTI) and in Wound Cases

*** B.L. Chaudhary**	Department of Microbiology, MGM Medical College and Hospital, Sector-18, Kamothe, Navi Mumbai- 410209, Maharashtra, India. * Corresponding author
Rakesh kumar Mukhia	Department of Microbiology, MGM Medical College and Hospital, Sector-18, Kamothe, Navi Mumbai- 410209, Maharashtra, India.
Rakesh pd sah	Department of Microbiology, MGM Medical College and Hospital, Sector-18, Kamothe, Navi Mumbai- 410209, Maharashtra, India.

ABSTRACT

Objective: To report trends of extended spectrum β-lactamase (ESBL), multidrug resistant (MDR) ESBL and AmpC producing isolates of K. pneumoniae from MGM Hospital, kamothe. Navi Mumbai.

Methods: Multidrug resistance, ESBL & AmpC production was tested by confirmatory methods as per Clinical Laboratory Standard Institute (CLSI) guidelines.

Results:- Out of 1670 sample (urine, pus and ET-secretion) 89(5.32%) klebsiella strain were isolated among them 57(64%) were MDR strains resistance to ceftizoxime 80%, cefuroxime 80%, tetracycline 78%, cefaperazone 75%,ceftazidime 70%. Among these isolates higher prevalence of ESBL and AmpC production was observed in ET-secretion 46.66% and 20% followed by urine 35% and 11.7% and pus 32%.and 8%.

Conclusion:- Detection for the ESBL and AmpC should be carried out as a routine it is simple and cost effective test to improve infections control practices to avoid irrational use of antibiotics and empirical regime should be revisited to prevent further resistance

KEYWORDS MDR, VAP, UTI Wound and Klebsiella species.

Introduction:

In 1883 Friedlander isolated a capsulated bacillus from the lungs of patient who died of pneumonia. This was named after him as Friedlander's bacillus. Later on this organism was given the generic name of Klebsiella, which is ubiquitously present and reported worldwide. Strains of Klebsiella are responsible for a wide variety of diseases in humans. These bacteria have become important pathogens in nosocomial infections.[1] In addition to being the primary cause of respiratory tract infections like pneumonia, rhinoscleroma, ozaena, sinusitis and otitis, it also causes infections of the alimentary tract like enteritis, appendicitis and cholycystitis. They are frequently associated with the infections of urinary tract, genital tract, and the eyes.[2] Extended spectrum β-lactamases (ESBLs) are plasmid-mediated bacterial enzymes that confer resistance to the penicillins (except temocillin), first-, second-, and third generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) and are inhibited by β-lactamase inhibitors such as clavulanic acid.[3] AmpC β-lactamases are Group I cephalosporinases that confer resistance to a wide variety of β-lactam antibiotics including alpha methoxy β-lactams such as cefoxitin, narrow and broad spectrum cephalosporins, aztreonam, and are poorly inhibited by β-lactamase inhibitors such as clavulanic acid.[4] During treatment with β-lactams, resistant mutants showing constitutive high levels of AmpC production are frequently selected leading to therapeutic failures. [5]. In India high prevalence of ESBL producing Klebsiella strains has been reported by various groups. Reported frequency of ESBL producing Klebsiella species from India ranged between 13 to 87%.[6,7,8,9,10,11,12]

Materials and Methods:-

Bacterial isolates:- A total of 89 consecutive, non-repeat clinical isolates of a Klebsiella strain was collected from Department of Microbiology, MGM Hospital Kamothe, Navi Mumbai, over a period of one year. (Feb 2012 to Feb 2013), both the outpatients and inpatients were included in the study. The isolates were obtained from different clinical specimens such as ET- secretion in case of ventilated associated pneumonia (VAP), 100 microliter of the homogenized sample was added to 100 ml of sterile normal saline definitive diagnosis of VAP, quantitative culture threshold was considered as 10^4 CFU/ml. Growth of any organism below the threshold were assumed to be due to colonization or contamination.[13,14] Urine for urine tract infection (UTI) and pus for wound cases by bacteriological conventional methods.[15]

Antimicrobial Sensitivity Testing: The antimicrobial sensitivity test of the isolates was carried out as described by the Kirby – Bauer disc diffusion method on Muller Hinton agar according to CLSI protocols. (16) Isolates were labelled as MDR if they were resistant to at least two classes of first line agents including ampicillin, trimethoprim- sulfamethoxazole, floroquinolones (ciprofloxacin and ofloxacin), gentamicin and cephalosporins (cephotaxime, ceftriaxone and ceftazidime).(17) The drugs tested were Ampicillin (10µg), amoxicillin clavulanic acid (20 / 10µg), piperacillin (100µg) piperacillin-tazobactam (100/10µg), cephotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg), cefpodoxime (10µg), gentamicin (10µg), amikacin (30µg) ciprofloxacin (5µg), tetracycline (30µg), chloramphenicol (30µg), trimethoprim-sulfamethoxazole (1.25 / 23.75µg) and imipenem (10µg). E. coli ATCC 25922 was used as control strains.

ESBL detection:- National Committee for Clinical Laboratory Standard (NCCLS) Phenotypic confirmatory combination disc diffusion test. A disc of ceftazidime (30µg) alone and ceftazidime + clavulanic acid (30µg/10) were placed at a distance of 25 mm centre to centre on a MHA plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37°C. An increase in inhibition zone diameter of ≥5mm for a combination disc versus ceftazidime disc alone confirmed ESBL producer. .Klebsiella pneumoniae ATCC 760063 was used as control strain.[18]

AmpC detection:- All isolates were tested for AmpC β- lactamase production on discs containing boronic acid. A disc containing 30 µg of cefoxitin and another containing 30 µg of cefoxitin with

400 µg of boronic acid was placed on the agar. Inoculated plates incubated overnight at 37°C. An organism demonstrating a zone diameter around the disk containing cefoxitin and boronic acid ≥5 mm than the zone diameter around the disk containing cefoxitin alone were considered an AmpC producer.[19]

Results and Discussion:-

Table no 1. Shows prevalence of Klebsiella species (5.32%) highest was shown by ET- secretion (20%) followed by pus (5.56%), and urine (3.08%). Whereas study by Kritu panta et all. shows prevalence of Klebsiella species (8.8%), urine (9.6%), pus (28.57%).[20] Study by David r park md et all. shows ET-secretion (2%).[21] Study by Olusola A.et all Shows prevalence of Klebsiella species in urine (14%), wound swab (30%).[22] Study by Bulent M. Ertugrul et all. shows ET-secretion (10.7%).[23] This study shows prevalence of Multidrug resistance (64.04%) highest was shown by ET-secretion (79.16%) followed by pus (60%) and urine (55.55%). This finding is much higher than a report by Shayam Kumar Mishra, (23.4%) in the same setting[24]. In case of wound swab by Girma Godema et all. was (69.6%).[25] Table 2 shows that Klebsiella isolates were from patients of which 27(61.7%) were males and 30(38.20%) were females.The male:female ratio was 0.9:1. In the study by A.O.Okesola et all. 30(34.1%) were males and 58(65.9%) were females. The male:female ratio was 0.6:1[26] Renuka Rampure et all. have reported the ratio of 199(51.8%) males and 185(48.2%) females. The male:female ratio was 1.07:1.[27]

Table 1) Prevalence of Klebsiella strain in ET-secretion, Urine and Pus.

Specimen	Total no specimen	No of Klebsiella isolated (%)	MDR strains(%)
Pus	675	38(5.62)	23(60.52)
Urine	875	27(3.08)	15(55.55)
ET-secretion	120	24(20)	19(79.16)
Total	1670	89(5.32)	57(64.04)

Table 2) Sex wise distribution of Klebsiella strains.

Specimen	Total no Klebsiella isolates	Klebsiella isolates from male patients (%)	Klebsiella isolates from female patients (%)	MDR strains (N=57)	
				Male (%)	Female (%)
Total	89	55(61.79)	34(38.20)	27(47.36)	30(52.63)

Table 3) Prevalence of ESBL and AmpC Producers among MDR Klebsiella species.

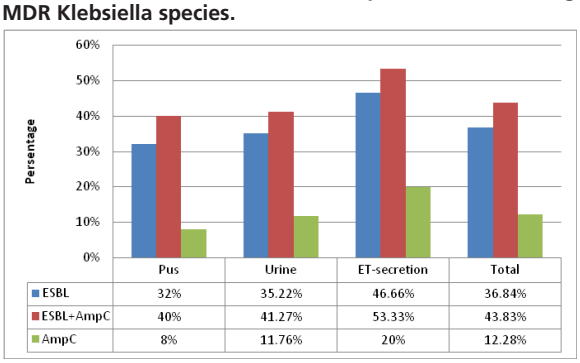

Table 3 Shows the prevalence of ESBL and AmpC as found in Klebsiella species isolated from the various clinical specimens. Prevalence of total ESBL was 36.84%, highest prevalence was seen in ET-secretion 7(46.66%), followed by urine 6(35.22%), pus 8(32%). Shamweel Ahmad et al. showed 10.4% prevalence of ESBL producing Klebsiella. The highest prevalence of ESBL was seen in ET-secretion (25%) followed by wound swabs (13.9%) and urine (9.2%).[28] Laghawe Avinash R. et all. showed 11.7% prevalence of AmpC in Klebsiella species.[29] Table 6 shows that out of 89 Klebsiella isolates the maximum resistance was observed in case pus to cephotaxime 80%, cefaperazone 75% and ceftazidime 70%. Urine was resistance to ceftizoxime 70%, tetracycline 78%, ampicillin/sulbactum 65%. and ET-secretion was cefuroxime 80%, cephotaxime 75% and cefaperazone 66%.Savita Jadhav et all. was shown maximum resistance to amoxicillin 82.59% followed by tetracycline 82%, cotrimoxazole 80%, ceftazidime 77.89% cephotaxime 57.97%, gentamicin 53.76%, ciprofloxacin 52.52%amikacin 41.78% and ceftriaxone 41.36%[30]

Table 4) Maximum sensitivity and resistance in MDR Klebsiella strains.

Specimen	Antibiotic showing maximum sensitivity (%)	Antibiotics showing maximum resistance (%)
Pus	OF (64%) GEN (60%) LOM (45%)	CTX (80%) CPZ (75%) CAZ (70%)
Urine	GF (70%) BA (65%) CH (65%)	CI (70%) TE (78%) AS (65%)
ET-secretion	PF (60%) OF (70%) LOM (60%)	CXM (80%) CTX (75%) CPZ (66%)

Conclusion:-

Antimicrobial resistance is a global concern not only because it kills but because it increases health costs and threatens patient care In this study, Klebsiella species was found to be the most predominant isolates as a cause of urinary tract infections, ventilated associated pneumonia and wound infection and also showed a very high prevalence of MDR. In case of wound shows resistance to ceftizoxime 80%, urine tract infection, tetracycline 78% and ventilated associated pneumonia, cefuroxime 80%. Among these isolates higher prevalence of ESBL and AmpC production was observed in ET-secretion 46.66% and 20% followed by urine 35% and 11.7% and pus 32%.and 8%. It is concluded that detection for the ESBL and AmpC should be carried out as a routine it is simple and cost effective test. MDR K. pneumoniae play a crucial role in spreading UTI, VAP and wound. Now, it is the need to improve infections control practices, avoid irrational use of antibiotics and empirical regime should be revisited to prevent further resistance.

REFERENCES

1. P. Nordamann, G. Cuzon and T. Naas "The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria", Lancet Infection Disease., 2009,9 (4):228-236. | 2. A.D. Dean, A.J. Dean, A.H. Burton and R.C.Dicker.Epi-Infoversion 5: a word processing database and statistics programme for epidemiology on microcomputers.VSD. Inc Stone Mountain Ga 1990. | 3. Paterson DL, Bonomo RA. Extended-Spectrum β-lactamase: a Clinical Update. Clinical Microbiology Reveiw 2005; 18: 657-86. | 4. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for b-lactamases and its correlation with molecular structure. Antimicrobial Agents Chemotherapy 1995; 39 : 1211-33. | 5. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R, et al. Evaluation of methods for AmpC beta-lactamase in Gram negative clinical isolates from tertiary care hospitals. Indian J Medical Microbiology 2005; 23: 120-4. | 6. Hansotia JB, Agarwal V, Pathak AA, Saoji AM 1997. Extended spectrum beta-lactamase mediate resistance to third generation cephalosporins in Klebsiella pneumoniae in Nagpur, Central India. Indian Journal Medical Research, 105: 160-165. | 7. Manchanda V, Singh NP, Goyal R, Kumar A, Thukral SS 2005 Phenotypic characteristics of clinical isolates of Klebsiella. | 8. Tankhiwale SS, Jalgaonkar VS, Ahamad S, Hassani U 2004. Extended spectrum β-lactamase in urinary isolates. Indian Journal of Medical Research, 120: 553-556. | 9. Shubha S, Ananthan S 2002. Extended spectrum β-lactamase (ESBL) mediated resistance to third generation cephalosporins among Klebsiella pneumoniae in Chennai. Indian J Med Microbiology, 20: 92-95. | 10. Shukla I 2004. Prevalence of extended spectrum β-lactamase in Klebsiealla pneumoniae in a tertiary care hospital. Indian Journal of Medical Microbiology, 22: 87-91. | 11. Mathur P, Kapil A, Das B, Dhawan B 2005. Prevalence of extended spectrum beta-lactamase producing Gram negative bacteria in a tertiary care hospital. Indian Journal of Medical Research, 122: 305-308. | 12. Clinical and Laboratory Standards Institute 2007. Performance Standards for Antimicrobial Testing. Seventeenth Informational Supplement. CLSI Document M 100- S17, 27: No. 1. | 13. Arindam Dey and Indira Bairy; KMC, Manipal. Incidence of multidrug-resistant organisms causing ventilator-associated pneumonia in a tertiary care hospital: A nine months' prospective study. Ann Thorac Med. 2007 Apr-Jun; 2(2): 52 57. | 14. Jakribettu RP, Boloor R. Characterisation of aerobic bacteria isolated from endotracheal aspirate in adult patients suspected ventilator associated pneumonia in a tertiary care centre in Mangalore. Saudi J Anaesth 2012; 6:115-9. | 15. Koneman EW, Allen, SD, Janda WM, Shcreckenberger PC Win WC. The Enterobacteriaceae. In: color atlas and textbook of diagnostic microbiology,5th ed. JB Lippincott Co: Philadelphia, 2006; 211-302. | 16. Clinical Laboratory Standards Institute. Performance standards for antimicrobial disc susceptibility testing. 14th informational supplement. 2004. | 17. Pokharel BM, Koirala J, Dahal RK et al. Multidrug resistant and extended spectrum β-lactamase producing Salmonella enterica from blood isolates in Nepal: surveillance of resistance and a search for a newer alternatives. International Journal of Infectious Disease. (2006) 10, 434-438. | 18. Cormican MG, Marshall SA, Jones RN. Detection of the extended spectrum β- Lactamases | (ESBL)- producing strains by the E-test ESBL screen. Journal Clinical Microbiology.1996; | 34: 11880-84. | 19. Kim YK, Pai H, Lee HJ, et al. J. Bloodstream infections which were caused by extended-spectrum β-lactamase producing Escherichia coli and Klebsiella pneumoniae in children: Epidemiology and clinical outcome. Antimicrobial Agents of Chemotherapy. 2002 ; 46 : 1-11. | 20. Kritu panta, Prakash ghimire, Shiba kumar rai, Reena kiran mukhiya, Ram nath singh, ganesh rai. antibiogram typing of gram negative isolates in different clinical samples of a tertiary hospital. Asian Journal of Pharmaceutical and Clinical Research Vol 6, Issue 1, 2013 | 21. David R Park MD. The Microbiology of Ventilator-Associated Pneumonia. Respiratory care • June 2005 ,Vol 50: NO 6 : 742- 747. | 22. Olusola A. Akingbade, I. J. Ogiogwa, Iheanyi O. Okonko, Phillip O. Okerentugba, Plasmid Profile of Isolated Klebsiella Species in a Tertiary Hospital in Abeokuta, Ogun State, Nigeria. World Applied Sciences Journal 21 (3): 371-378, 2013 | 23. Bulent M. Ertugrul et all .Ventilator-associated pneumonia in surgical emergency intensive care unit. Saudi Med J 2006; Vol. 27 (1): 52-57 | 24. Shyam Kumar Mishra et all. Drug Resistant Bacteria are Growing Menace in a University Hospital in Nepal. American Journal of Epidemiology and Infectious Disease, 2014, Vol. 2, No. 1, 19-23 | 25. Girma Godebo1, Gebre Kibru2* and Himanot Tassew Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. Annals of Clinical Microbiology and Antimicrobials 2013, 12:17 | 26. A.O.Okesola et al. Prevalence of extended-spectrum β-lactamase producing Klebsiella in a tertiary care hospital in South West Nigeria. International Journal of Pharmacology Biomedical Sciences 2012, 3(4), 148-151 ISSN No: 0976-5263 | 27. Renuka Rampure et al Prevalence of MDR-ESBL producing Klebsiella Pneumonia isolated from clinical Samples. J. Microbiol. Biotech. Res., 2013, 3(1):32-39 | 28. Shamweel Ahmad et all. Prevalence, Antibiotic Susceptibility Pattern and Production of Extended-Spectrum b-Lactamases Amongst Clinical Isolates of Klebsiella pneumoniae at Armed Forces Hospital in Saudi. Arabian Journal of the College of Physicians and Surgeons Pakistan 2009, Vol. 19 (4): 264-265. | 29. Laghve Avinah et all. The simultaneous detection of ESBL and AmpC β lactamases in Gram negative bacilli. Indian journal of Medical Microbiology. 2005; 23:120-124. | 30. savita jadhav et all. Increasing incidence of multidrug resistance klebsiella pneumoniae infections in hospital and community settings. International Journal of Microbiology Research ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 4, Issue 6, 2012, pp.-253-257 |