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Antimicrobial Susceptibility Patterns of Clinical isolates of Gram-negative Pathogens from a Teaching Hospital, Pondicherry.

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ABSTRACT

Antimicrobial resistance is a global emerging problem in the community and in hospitals. The present study was undertaken to evaluate the antimicrobial susceptibility patterns of common gram negative organisms isolated from hospital specimens. Antimicrobial susceptibility data of organisms isolsted from different samples were analyzed in the department of pharmacology and Microbiology at Sri Manakula Vinayagar Medical College Hospital, Pondicherry over eight months period. Out of 5381 specimens, the culture positive specimens were pus 60%, Cerebrospinal fluid 31.5%, urine 26%, synovial fluid 25%, swabs 25%, ascitic fluid 23.2%, sputum 18.6%, stool 18%, pleural fluid 15.5%, blood 13%, & others 27.7%. Gram negative bacteria accounted for 62% of isolates. The main species were E.coli (52.6%), Klebsiella spp. (21.3%), Pseudomonas spp. (18%) and Proteus spp. (4%). Maximum susceptibility of E. coli was observed with Imepenam (99.7%), Pipercillin+ Tazobactam, (97% each) followed by Meropenem (95%), Nitrofurantoin (92%) and Amikacin(84%) ; of Klebsiella spp. and Proteus spp. to Meropenem, Pipercillin + Tazobactam, (100% each) and of Pseudomonas spp. to Imepenam (82.6%), Aztreonam, Ciprofloxacin (80%each) followed by Tobramycin (77.7%). High level of resistance was observed with Amoxycillin + Clavulanic acid (80 -90%), Ampicillin (65 – 95%), Co-trimoxazole (70 – 90%), Ciprofloxacin (60 – 90%). Based on the above study Imepenam, Pipercillin+ Tazobactam, Meropenem were the effective antimicrobials for E. coli, Proteus spp. and Klebsiella spp. and Imepenam, Aztreonam, Ciprofloxacin and Tobramycin for Pseudomonas spp.
Keywords: Gram negative bacteria, Antimicrobials, Susceptibility, Resistance.

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INTRODUCTION

Infection and antimicrobial resistance are global concern especially in developing nations, including India, where the burden of infectious disease is high and healthcare spending is low. The World Health Organization and the European Commission have recognized the importance of studying the emergence and the determinants of antibiotic resistance and the need for strategies for its control. [1, 2] The emerging problem of antimicrobial resistance, especially among Gram-negative bacteria has become a serious threat to public health. [3] This antimicrobial resistance often results in increased morbidity, mortality and costs of treatment. Preventing the emergence and dissemination of resistant organisms and manage them efficiently is critical for control of hospital infections. Appropriate antimicrobial stewardship that includes optimal selection of drug, dose and duration of treatment, as well as control of antibiotic use, will prevent or minimise the emergence of resistance among microorganisms [4]. In addition it is widely held that surveillance of antimicrobial susceptibility is fundamental to combat the emergence of resistance. [5] Awareness of local antimicrobial susceptibility pattern of gram negative bacteria is essential when the patient is treated empirically. Moreover the knowledge of likely prevalent strains along with their antimicrobial resistance pattern will help in better management of patients and framing the antibiotic policy. [6]

Hence, this study was undertaken to evaluate the current status of antimicrobial susceptibility pattern of the common gram negative bacterial isolates from various clinical specimens in a teaching hospital of Pondicherry.

MATERIALS AND METHODS

This present study was conducted in the Department of Pharmacology and Microbiology of Sri Manakula Vinayagar Medical College Hospital, Pondicherry. Retrospective analysis of clinical samples of urine, blood, pus, swabs, cerebrospinal fluid(CSF), ascitic fluid(AF), synovial fluid(SF), pleural fluid(PF), stool, sputum etc., during the period from January 2012 to August 2012 were studied. These samples were processed for culture and sensitivity by standard methods. [7] All significant isolates were identified by standard procedures and their antimicrobial susceptibility was tested by Kirby Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute (CLSI) recommendations. [8] Appropriate control strains were used for quality control. The antimicrobials used for the isolates were Ampicillin, Amoxycillin+clavulanic acid, Cotrimoxazole, Ticarcillin, Piperacillin, Piperacillin +Tazobactum, Cefazolin, Cefotaxime, Ceftazidime, Ceftriaxone, Imipenam, Meropenam, Aztreonam, Nalidixic acid, Nitrofurantoin, Norfloxacin, Ciprofloxacin, Levofloxacin, Gentamicin, Amikacin, Tobramicin and Tetracycline . The data were entered in Microsoft excel and analyzed using Statistical package for the social sciences (SPSS) 3.4.3 software and the results were expressed in percentages.

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RESULTS

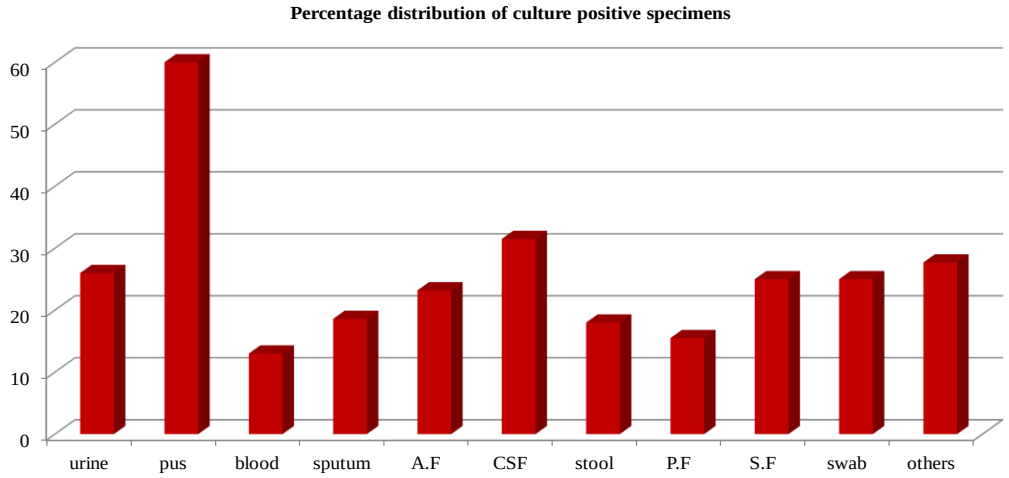
Overall 5381 biological specimens were analysed and 1485 bacterial isolates were recovered from different range of clinical specimens of both inpatients and out patients. (Table 1. Figure 1&2) Among them 586(39.46%) were gram positive and 899(60.53%) were gram negative bacterial isolates. The most common bacteria isolated were E.coli 483(54%) followed by Klebsiella spp.196 (22%), Pseudomonas spp.167 (18%), Proteus spp. 38(4%), Citrobacter 8 (0.8%) and others 7(0.7%). (Figure 3)

Table 1: Frequency and Percentage of processed specimens during the study period (n= 5381)

Specimen	urine	blood	pus	sputum	A.F	CSF	stool	P.F	S.F	Swab	others
Frequency	2486	1002	794	661	43	19	160	45	12	87	72
Percent	46.2	18.6	14.7	12.3	0.79	0.35	2.9	0.8	0.2	1.6	1.3

(P.F –Pleural fluid, A.F – Ascitic fluid, S.F – Synovial fluid, CSF – Cerebrospinal fluid)

Figure1. Percentage distribution of culture positive specimens



(P.F –Pleural fluid, A.F – Ascitic fluid, S.F – Synovial fluid, CSF – Cerebrospinal fluid)

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Figure 2: Frequency distribution of culture positive and negative specimens

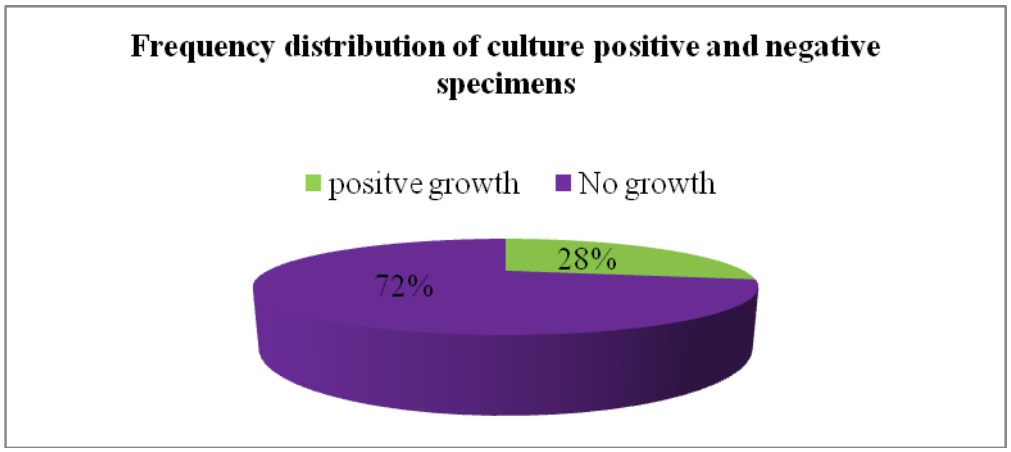
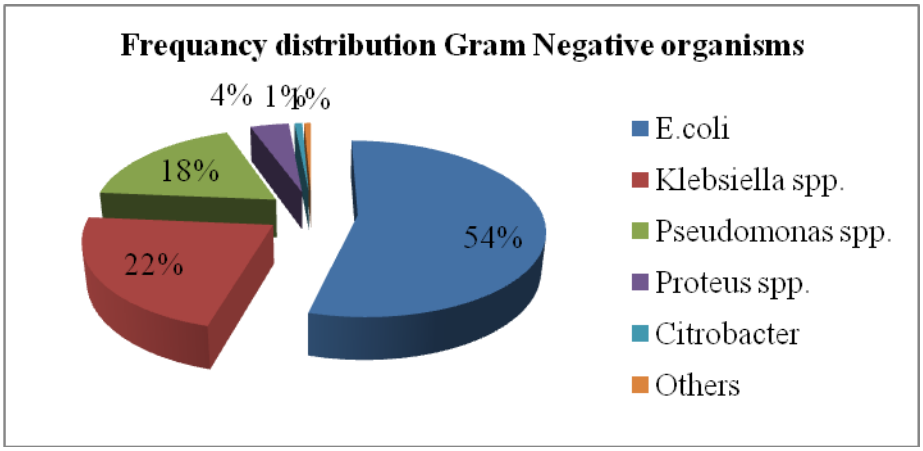


Figure 3: Frequency distribution of Gram Negative organisms



In our study, the common gram-negative pathogens from urine and blood were Escherichia coli (82% and 2.3%respectively) and Klebsiella spp. (35% and 2.6% respectively). Pseudomonas spp. was mostly isolated from pus (64%). The common gram-negative bacteria from sputum were Klebsiella and Escherichia coli (27% and 2.1% respectively). (Table2)

Table 2: The microbial spectrum of common gram-negative bacteria isolated from different clinical specimens.

Specimen type	E.coli (%)	Klebsiella spp. (%)	Pseudomonas spp. (%)	Proteus spp. (%)
Urine	379(82)	66 (35)	20 (16.6)	18 (41.8)
Pus	45(9.7)	51(27)	77 (64)	23(53.4)
Sputum	10(2.1)	51(27)	3 (2.5)	-
Blood	11(2.3)	5 (2.6)	2(1.6)	-
Swabs	7(1.5)	3 (1.5)	6 (5)	-
Others	9(1.9)	12(6.3)	12(10)	2(4.6)

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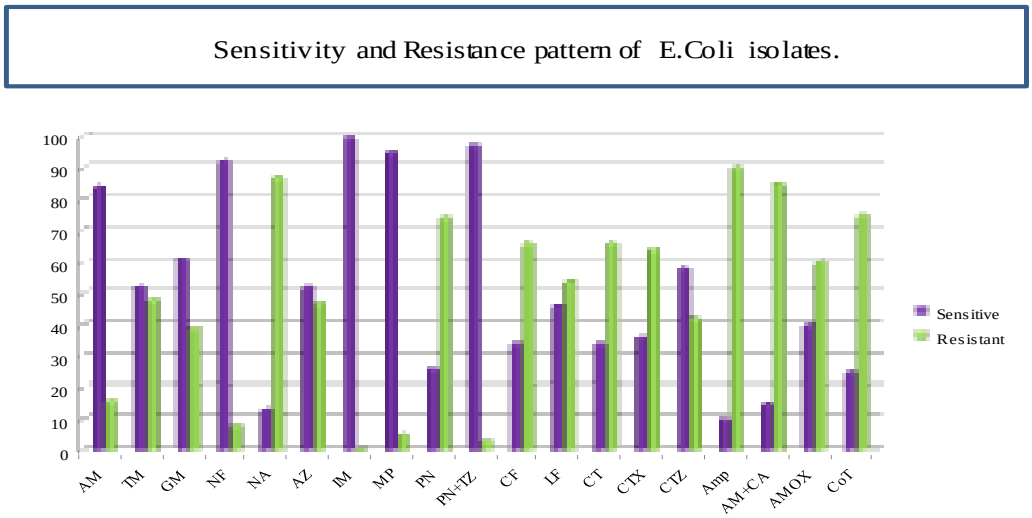
E.coli

The sensitivity and resistance pattern of E.coli isolates to different antimicrobials are represented in the Figure 4. High level of sensitivity was seen with Imipenem(99.7%), Piperacillin+Tazobactum(97%) , Meropenam(95%), Nitrofurantoin(92%), Amikacin (84%), followed by Ceftazidime(58%), Gentamicin(57%) , Aztreonam(52%), and Tobramycin(51%).

Figure 4: Sensitivity and Resistance pattern of E.coli isolates.



The sensitivity and resistance shown in the figure is the percentage

(Amp- Ampicillin, AM+CA-Amoxycillin+clavulanic acid, CoT- Cotrimoxazole, AMOX-Amoxycillin, PN-Piperacillin, PN+TZ-Piperacillin +Tazobactum, CT-Cefotaxime, CTZ-Ceftazidime, CTX-Ceftriaxone, IM-Imipenam, MP-Meropenam, AZ-Aztreonam, NA-Nalidixic acid, NF-Nitrofurantoin, CF-Ciprofloxacin, LF-Levofloxacin, GM-Gentamicin, AM-Amikacin, TM-Tobramicin).

We observed very high rate of resistance with Ampicillin(88%), Nalidixic acid(86%), Amoxycillin/ clavulanic acid(84%), Cotrimoxazole(74%) and Piperacillin(72%). The other drugs showed resiatance rates less than 70%.

Klebsiella Spp.

The sensitivity of Klebsiella species to both Carbapenems, Imipenem and Meropenem, was 97.6% and 100% respectively; to Aztereonam a Monobactam was 53.3%.The percentage sensitivity to Cefotaxime and Ceftrixone, Ceftazidime were 59.7% and 55.3 % respectively. Among the Penicillin group of antimicrobials tested high level of sensitivity was seen with Piperacillin + Tazobactam (100%). High level of resistance was seen with Ampicillin 94.8%,

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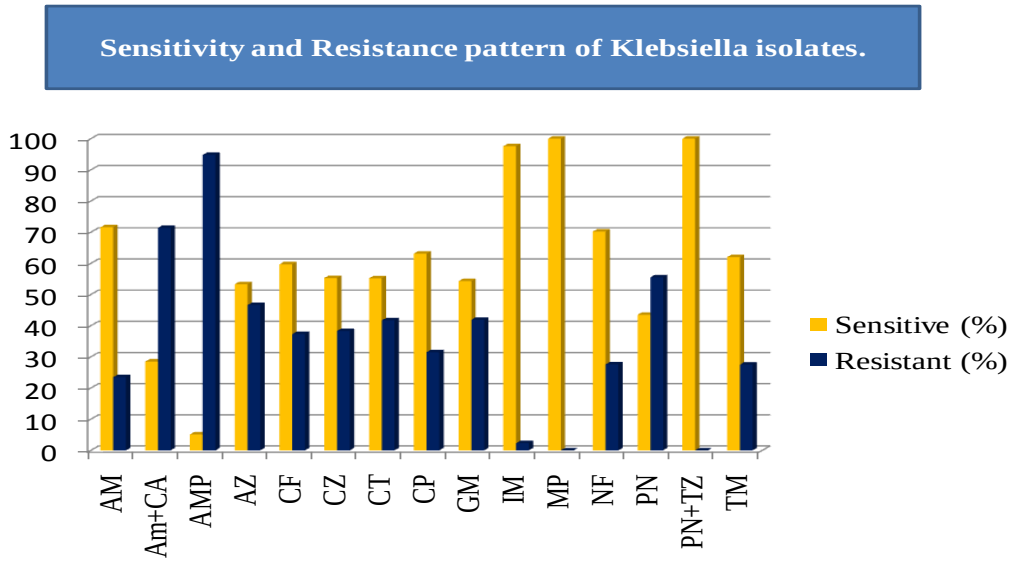
Ticarcillin 94.2%, Amoxycillin+ Clavulanic acid (71.4%), Penicillin 66.6% and Piperacillin55.5%.(Figure 5)

Figure 5: Sensitivity and Resistance pattern of Klebsiella isolates.



The sensitivity and resistance shown in the figure is the percentage

(AMP- Ampicillin, AM- Amoxycillin AM+CA-Amoxycillin+clavulanic acid, PN-Piperacillin, PN+TZ-Piperacillin +Tazobactum, CT-Cefotaxime, CTZ-Ceftazidime, CTX-Ceftriaxone, IM-Imipenam, MP-Meropenam, AZ-Aztreonam, NF- Norfloxacin, CF-Ciprofloxacin, GM-Gentamicin, AM-Amikacin, TM-Tobramicin).

Proteus spp

Among the Proteus isolates, high level of sensitivity (100%) was seen with Piperacillin + Tazobactam, Meropenam and Aztreonam whereas for Imepenam 94.7% and Piperacillin74%. The sensitivity rates to Cefotaxime, Ceftrixone and Ceftazidime for Proteus isolates were 81.2%, 66.6% and 52% respectively. High level of resistance was seen with Amoxycillin + Clavulanic acid (100%), Ciprofloxacin 87.5% and Tobramycin 60%.(Figure 6)

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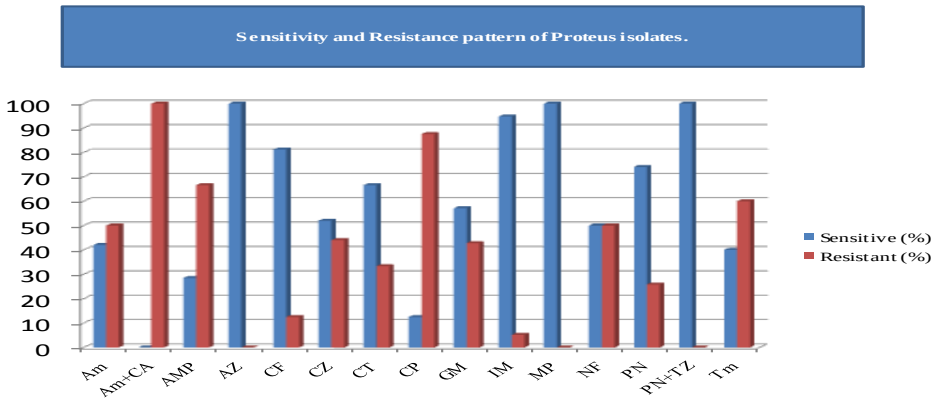
Figure 6: Sensitivity and Resistance pattern of Proteus isolates.



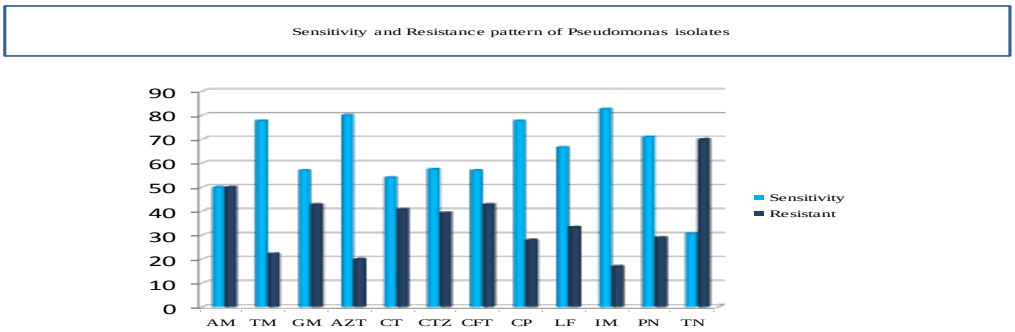
The sensitivity and resistance shown in the figure is the percentage

(Am- Ampicillin, AM+CA-Amoxycillin+clavulanic acid, AMP-Amoxycillin PN-Piperacillin, PN+TZ-Piperacillin +Tazobactum, CF-Cefotaxime, CTZ-Ceftazidime, CTX-Ceftriaxone, IM-Imipenam, MP-Meropenam, AZ-Aztreonam, NF- Norfloxacin, CP-Ciprofloxacin, GM-Gentamicin, Tm-Tobramicin).

Pseudomonas spp

Pseudomonas isolates had susceptibility rates of 82.6% to Imepenem, 80% to Aztreonam and Ciprofloxacin, 77.7% to Tobramycin, 71% to Piperacillin, 57.5% to Ceftazidime, 57.1% to Gentamicin and Ceftriaxone. The resistance rates for Ticarcillin and Amikacin are 69.2% and 50% respectively. (Figure 7)

Figure 7: Sensitivity and Resistance pattern of pseudomonas isolates.



The sensitivity and resistance shown in the figure is the percentage

(TN-Ticarcillin, PN-Piperacillin, CT-Cefotaxime, CTZ-Ceftazidime, CTX-Ceftriaxone, IM-Imipenam, AZT-Aztreonam, CP-Ciprofloxacin, LF-Levofloxacin, GM-Gentamicin, AM-Amikacin, TM-Tobramicin).

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DISCUSSION

The present study provided an outlook on the prevalence and the antibiogram of the common gram negative pathogens which were isolated in a teaching Institution at Pondicherry. Among the gram negative organisms E. coli was the most predominant (60.53%) and was the most common isolate from urine specimens examined [9]. The predominance of gram negative pathogens is similar to a study conducted in Chennai [10]. We also observed the rate of isolation of Klebsiella and Proteus isolates were similar to a study conducted in Maharashtra. [11]. *However Pseudomonas* isolation in our study was high (18%) compared with study conducted by *Prasad Niranjan Gunjal et al. in Maharasthra [*11].

Beginning with the introduction of penicillin's half a century ago, the β-lactams have remained the largest antibiotic class of clinical relevance. However they are rapidly hydrolyzed by broad spectrum β-lactamases that are found with increasing frequency in clinical isolates of these gram negative bacteria. Several reports have indicated an increased resistance to Ampicillin and Amoxicillin (80-100%) against gram negative organisms.[10,12,13] Along with β-lactamase inhibitors the spectrum of activity of Aminopenicillins can be extended. But it was not rewarding in our study.

In our study, results with Cephalosporins and Aminoglycosides sensitivity pattern are similar to other studies conducted in India earlier. [14, 15] Overall resistance to various Cephalosporins was also high except few of them and could be due to the over use of these drugs especially third generation Cephalosporins in hospitals and also due to production of ESBLs by the gram negative bacteria involved. Several studies have demonstrated the relationship between the use of third generation Cephalosporins and acquisition of ESBL-producing organisms [16-18]. The Carbapenem group of antimicrobials which are usually resistant to most of the β-lactamases, have a broader spectrum of activity than other β-lactam antibiotics against gram negative organisms. Most of the strains of E. coli, Klebsiella, Pseudomonas and Proteus in our study were inhibited by Carbapenems (80 – 100%). Hence, it would be the better choice to use Imipenem or Meropenem for empirical treatment of serious infections caused by these gram negative bacteria. Their higher sensitivity rates to Imepenam, Meropenam was supported by another study. [19]

Our study also revealed that Fluoroquinolones used in our hospital have reduced activity against Escherichia coli, Klebsiella and Proteus. [20] However surprisingly increased sensitivity rate is seen with this group against Pseudomonas isolates (78%) which is supported by another study. [21]

The high levels of antimicrobial resistance in gram-negative bacteria could be due to genetic factors, increased usage of higher antibiotics/ inappropriate use of antibiotics. Therapeutic management of infection involves consideration of susceptibility-resistance patterns, pharmacokinetic profile, prophylactic/combined antibiotic therapy, host-defense mechanisms, local factors and adverse reactions of the drug etc. There is a need to inculculate rational use of antimicrobials and to restrain the further development of drug resistance. In

addition, regular antimicrobial susceptibility surveillance is essential for monitoring drug resistance patterns. An effective and better patient management necessitates having an Institutional antibiotic policy in addition to national antibiotic policy in order to prevent emergence of drug resistance.

CONCLUSION

Based on the present study the use of antimicrobials like Semisynthetic Penicillins (Piperacillin) with beta lactamase inhibitors (Tazobactam), Aminoglycosides (Amikacin, Tobramycin, Gentamicin) and third generation Cephalosporins (Ceftriaxone, Cefotaxime and Ceftazidime) are recommended for the treatment of common gram negative pathogens. Further Imipenem, Meropenem and Aztreonam should be considered as reserve drugs for the treatment of severe nosocomial infections to avoid emergence of resistance strains. Moreover periodic monitoring of antimicrobial susceptibility patterns of common bacterial isolates and revising the antibiotic policy appropriately is recommended.

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