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Research Article

A STUDY OF OCCURRENCE OF ANTIBIOTIC RESISTANCE REPORTED AGAINST *KLEBSIELLA* SPP. IN A TERTIARY CARE HOSPITAL IN ASSAM, INDIA

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ABSTRACT
A prospective study was conducted in the Department of Microbiology, AMCH (Assam Medical College and Hospital), where pus, urine, blood, sputum and wound swab samples were collected of patients from new-born to 85 years of age and cultured on MacConkey Agar and Blood agar media plates at 37^0 C for 18-24 hrs.(overnight). A total of 100 samples positive for *Klebsiella* spp. were collected. Bacteria were identified by their culture, morphological and bacteriochemical characteristics using standard microbiological methods. Antibiotic sensitivity was tested using conventional disc-diffusion method by Kirby-Bauer technique using Mueller-Hinton Agar plates. A total of 100 *Klebsiella* spp. positive samples were studied with 9 antibiotics as mentioned later. The data on percentage antibiotic resistance out of 100 samples were Amoxycillin-Clavulanic acid=75%, Ceftriaxone=69%, Cefotaxime=65%, Piperacillin-Tazobactam=57%, Amikacin=47%, Gentamycin=46%, Ciprofloxacin=41%, Ofloxacin=32% and Imipenem=0%.Increased antibiotic resistance of *Klebsiella* spp .as seen in the study conducted increases concern over formulation of proper, national antibiotic policy and its proper implementation along with issues of "Antibiotic Stewardship".
Key words: MacConkey Agar, *Klebsiella* spp., disc-diffusion, Kirby-Bauer, Muellar-Hinton Agar, "Antibiotic Stewardship".

INTRODUCTION

Antibiotic chemotherapy had been critical in the fight against infectious disease and a leading cause for dramatic rise in average life expectancy since the middle of the twentieth century–ever since the first antibiotic penicillin came into clinical usage in 1946. However the emergence of resistance among bacteria to these drugs in varying degrees of severity and combinations threatens to erode the significant gains made in combating infections[1].Today every major class of antibiotic is associated with the emergence of significant resistance. This serious development is ever present with each new antimicrobial agent and threatens the end of antimicrobial era[2].

Resistance to antibiotics is the ability of bacteria to survive exposure to an antibiotic to which they were previously susceptible .Multidrug resistant bacteria is able to resist antibiotics of a wide variety of structure and function.

The global problem of antibiotic resistance is particularly pressing in developing countries where the infectious disease burden is high and there are cost constraints on testing to identify resistant infections and using newer and more expensive agents to treat them. In 2007, the prevalence of MRSA (Multidrug Resistant *Staphylococcus aureus*) ranged from 27.4 to 62.4 % in the different census regions of the United States. Pneumococcal isolates were resistant to Penicillin in France, Spain, North Korea; resistance to erythromycin varies from very low levels in Sweden to very high levels in South Korea; resistance to fluoroquinolones was found in Hong-Kong and to Macrolide antibiotics in South Africa. By July, 2010, 58 countries had reported at least one case of XDR-TB[1].

Klebsiella spp. refers to the different species under the genus *Klebsiella*; the genus is defined as containing gram-negative, non-motile usually encapsulated rod-shaped bacteria of the family *Enterobacteriaceae*. Under the genus *Klebsiella*, the species are as- *Klebsiella oxytoca, Klebsiella pneumoniae, Klebsiella terrigena, Klebsiella planticola*, etc. They are opportunistic pathogens and can cause severe diseases like septicaemia, pneumonia, UTI (Urinary Tract Infection) and soft-tissue infections. It is estimated that *Klebsiella* spp. causes 8% of all nosocomial bacterial infections in the United States and in Europe. No great geographical variations in frequency have been noted. In the United States, *Klebsiella* accounts for 3-7% of all nosocomial bacterial infections[3].

Microorganisms exhibit resistance to antibiotics mainly by – a) drug inactivation by producing enzyme, b) prevention of drug accumulation in the bacterium, c) modification/protection of the target site ,d) use of alternative pathways for metabolic/growth requirements, e) Quorum sensing[4].

MATERIALS AND METHODS

Sample collection and analysis

A prospective type of study was conducted in the Department of Microbiology, Assam Medical College and Hospital, Dibrugarh between July, 2011 and June, 2012. Fresh specimens of pus, urine, sputum, blood and wound swabs were collected from the patients between new-born to 85 years of age (sent for their specimen examination by different clinical departments);100 samples were collected where *Klebsiella* spp. had grown; this included 16 pus, 23 urine, 3 blood, 40 sputum, 18 wound swab samples respectively. Bacterial inoculation and culture from these specimens were carried on MacConkey and Blood Agar Media plates, incubated at 37^0 C for 18-24 hrs. (overnight incubation) as per CSLI (Clinical Laboratory Standard Institute) Standards[5]. Bacteria were identified by culture, morphological and biochemical characteristics of bacteria using standard microbiological methods[3].

Hi-Media kits, supported by Hi-Media kits' manufacturer's instructions were followed to identify the different organisms under *Klebsiella* spp.

Antibiotic sensitivity testing

Antibiotic sensitivity testing was carried out using conventional disc-diffusion method by KIRBY-BAUER technique using Mueller-Hinton Agar plates as described by

the Clinical Laboratory Standard Institute (CLSI)[5-6]. Antimicrobial agents (disks) tested and reported were obtained from Hi-Media labs., Mumbai, India.

Interpretation of antibiotic susceptibility

Susceptibility testing of the bacterial isolates by disc-diffusion tests against different classes of antimicrobial agents was reported in the form of diameter of inhibition zone and compared with standard charts obtained from Hi-Media labs., Mumbai.

Table 1: Antibiotic resistance pattern of *Klebsiella* spp. (As found from different specimens)

Antibiotic used	Total no. of samples(n)=100		% of antibiotic resistance
	Sensitive	Resistant	
Amoxicillin-clavulanic acid	25	75	75
Ceftriaxone	31	69	69
Cefotaxime	35	65	65
Piperacillin-tazobactam	43	57	57
Amikacin	53	47	47
Gentamycin	54	46	46
Ciprofloxacin	59	41	41
Ofloxacin	68	32	32
Imipenem	100	0	0

Table 2: Multiple Antibiotic Resistance (MAR) Index (as per findings)

No. of antibiotic resistant	No. of specimens	MAR index
0	0	0
1	12	0.11
2	12	0.22
3	16	0.33
4	12	0.44
5	13	0.55
6	17	0.66
7	11	0.77
8	7	0.88
9	0	1

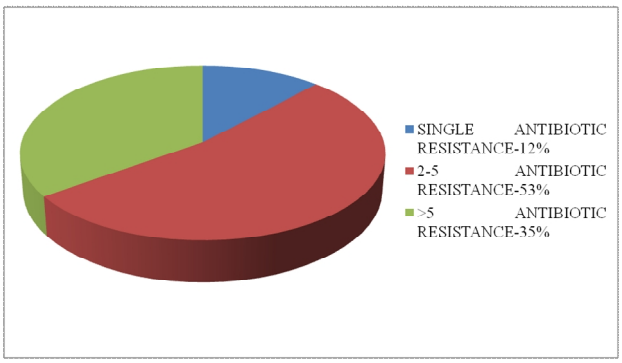

Figure 1: Pie-chart showing antibiotic resistance of *Klebsiella* spp. (as per findings)

RESULTS

Table 1 shows antibiotic resistance pattern of *Klebsiella* spp. (as found from different specimens); the data on percentage antibiotic resistance of the 100 *Klebsiella* spp. positive samples studied with the 9 antibiotics used (as shown in the table 1) are: Amoxicillin-Clavulanic acid=75%, Ceftriaxone=69%, Cefotaxime=65%, Piperacillin-Tazobactam=57%, Amikacin=47%, Gentamicin=46%, Ciprofloxacin=41%, Ofloxacin=32% and Imipenem=0%.The samples show highest degree of resistance with Amoxicillin-Clavulanic acid (75%) and the lowest degree of resistance with Imipenem (0 or nil).

Figure 1 shows that 12% samples were resistant to a single antibiotic (any), 53% of the samples were resistant to 2-5 no. (number) of antibiotics (any) and 35% of the samples were resistant to more than 5 no. (number) of antibiotics (any) respectively;

Table 2 shows the MAR (Multiple Antibiotic Resistance) index of the different samples or specimens;

MAR Index was found to be 0.11, 0.22, 0.33, 0.44, 0.55, 0.66, 0.77, 0.88 in 12, 12, 16, 12, 13, 17, 11 and 7 number of specimens respectively. MAR equal to 0 (zero) /1 was found in no or zero number of specimen.

DISCUSSION

In our present study, we found that *Klebsiella* spp. showed the highest degree of antibiotic resistance, with the Penicillin and Cephalosporin groups of drugs: highest with Amoxicillin –Clavulanic acid; less so with the Aminoglycosides and Fluoroquinolones and no resistance with Imipenem.

MAR Index of 88 out of 100 Klebsiella spp. positive samples were found to be more than 0.2.

MAR (MULTIPLE ANTIBIOTIC RESISTANCE) is a tool that gives an indirect suggestion of the probable source(s) of an organism. According to previous workers, MAR index >0.2 indicates that an organism must have originated from an environment where antibiotics are often used[7].

Determination of MAR Index

The MAR index was determined for each isolate by dividing the no. of antibiotics to which the isolate is resistant by the total no. of antibiotics tested[8].

Klebsiella spp. are Gram-negative, non-capsulated bacteria; they are ubiquitous in nature. *Klebsiellae* probably have two common habitats, one being the environment where they are found in surface water, sewage and soil and on plants and the other being the mucosal surfaces of mammals. Until recently, only *K.pneumoniae* and *K.oxytoca* had been considered pathogenic but now *K.terrigena* and *K.planticola*, formerly regarded as "environmental" *Klebsiella* species have been demonstrated to occur in human clinical specimens. Typically *Klebsiella* infections are nosocomial; targeting mainly hospitalised, immunocompromised patients with underlying diseases. Pathogenicity factors of *Klebsiella* include capsular antigens, pili, siderophores, lipopolysaccharides, capsular polysaccharides[3].

Klebsiella participates in exchange of plasmids with other *Enterobacteriaceae* which is presumed to be the basis for its antibiotic resistance[9].

Antimicrobial drug resistance is not a new phenomenon; however the current magnitude of the problem and the speed with which the new resistance phenotypes have emerged elevates the public health significance of this issue. The societal and financial costs of treating antimicrobial resistant infections place a significant human and economic burden on society as individuals infected with drug resistant organisms are more likely to remain in the hospital for a longer period of time and have poor prognosis. In addition, the scarcity of new antimicrobial agents and the dearth of new agents in the drug development pipeline limit treatment options, particularly for patients with infections caused by multi-drug resistant (MDR) organisms, which occur mainly in health care settings[10].For the drug industry it, represents diminished marketability of current products[1].

As a result, it has become very important to take quick, effective steps against the emergence of antimicrobial resistance.

CONCLUSION

Finally, it can be said that *Klebsiella* spp. is also showing its increased resistance to Fluoroquinolones and other groups of drugs, besides penicillin group of antibiotics. Rational and judicious use of antibiotics can prevent the development of

antibiotic resistance by this group of organisms. Formulation of a proper national antibiotic policy for rational use of drugs and regular monitoring of progress in the implementation of policies and guidelines on issues related to antibiotic resistance has become inevitable, which could help in the modern day scenario of emerging antibiotic resistance.

Effective application of issues of "Antibiotic Stewardship" can limit the increasing antibiotic resistance as:

a) Avoiding indiscriminate use of antibiotics by ensuring their indication, dose & duration of treatment.

b) Restricting use of antimicrobial combinations to appropriate circumstances.

c) Constant monitoring of resistance patterns in a hospital or community, changing empirical drug therapy when needed. Proper infection control practises in hospitals should be made compulsory.

d) Restricting drug use, e. g .limit usage of newest member of a group of antimicrobials so long as the current drugs are effective[11].

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