

ANTIBIOTIC SENSITIVITY PATTERN OF ISOLATED MICROORGANISMS FROM VARIOUS CLINICAL SPECIMENS AT TERTIARY HEALTH CARE CENTRE

SUMAN KUMARI A^{#1} LOUIS COJANDARAJ¹

^{#1} Department of Medical Laboratory Sciences, School of Physiotherapy and Paramedical Sciences, Lovely Professional University, Phagwara, Punjab, 144411.

#Corresponding Author:

Suman Kumari
Assistant Professor,
Department of Medical Laboratory Sciences
Lovely Professional University
Suman.25115@lpu.co.in

ABSTRACT

Multiple Drug Resistance in microorganisms is one of the biggest glitches faced by medical practioners for treatment of microbial infections such as urinary tract infection, wound infection, skin infection etc. It is because of use of undefined antibiotic policy, prescribing potent antibiotics and using empirical method of treatment without culture, diagnosis and identification of strain. To overcome this problem, a standardized method of antibiotic treatment should be followed after performing culture, isolation and identification of microorganisms, biochemical test and standardized sensitivity pattern. This study was conducted at SGHS hospital, microbiology laboratory involving review of patient medical laboratory records of microorganisms and the sensitivity pattern of microorganisms isolated from versatile clinical specimens such as urine, pus, tracheal secretions, body fluids etc., collected from the patients of inpatient and outpatient department of the SGHS hospital, Mohali during the period Jan 2017 to March 2017. Out of 1142 clinical specimens, 319 were isolated. *E.coli* was the most frequent isolate (48.9%), followed by *Klebsiella pneumoniae* (23.8%), *Acinetobacter spp.* (9.40%), *Pseudomonas aeruginosa* (7.21%), *Staphylococcus aureus* (6.58%), *Candida spp.* (2.50%), *Proteus spp.* (0.94%) and *Enterococcus spp.* (0.62%). Gram negative isolates were most sensitive to Nitrofurantoin, Imipenem and Amikacin while resistant to Ampicillin and cephalosporins. Gram positive isolates were sensitive to Vancomycin and Doripenem while resistant to Ampicillin. This study will guide the clinicians in choosing suitable antimicrobial drug to treat infections as well as in prevention of development of resistance to drugs in pathogens.

Keywords: Antibiotic sensitivity, Multiple Drug Resistance, *E.coli*, Colistin, Nitrofurantoin.

Introduction:

Microbial infections have proved life threatening since pre-antibiotic era.[1] Many infections have spread worldwide, pandemic or epidemic outbreaks in this era. But after discovery of "the magic bullet" penicillin in 1943, the first antibiotic, it gave huge relief to clinicians and cured many life threatening infections. [2] A serious threat to public health is occurrence of antimicrobial resistance in pathogenic microorganisms. [3] This AMR results in high health care cost, failure to respond in treatment and high death rates. Moreover, in some cases resistant microorganisms may lead to development of untreatable infections like neonatal sepsis [4]. The crude infectious disease mortality rate in India today is 416.75 per 100,000 persons (author calculations based on World Bank data & the global burden of disease, 1990) [5]. Microorganisms develop resistance to drugs because of misuse of drugs, self-medication, irrational use of drugs, undefined antibiotic policy etc. Resistance has appeared even newer and more effective antibiotics such as Carbapenem. [6] The process of development of antimicrobial resistance is very slow. But repeated and misuse of antibiotics modifies the path physiology mechanism in microbes for persistence. [7] The prevalence of AMR pattern differs between countries and pathogenic microorganisms. The AMR configuration of bacterial isolates keep altering and progressing with time and place. [8] To overcome this problem, there is a need to educate and create awareness among public and health care professionals regarding infection control measures and AMR.

Materials and Methods:

This study was conducted in Microbiology Department of Sri Guru Har Krishan Sahib eye and super speciality hospital, Sohana, Mohali during the period January 2017 to April 2017.

A total of 1142 clinical specimens were collected and the specimens targeted for this study was urine, pus/wound swab, sputum, tracheal secretions, BAL, body fluids (CSF, Peritoneal fluid, Pleural fluid, Ascitic fluid, Cervical fluid, Bile, Vitreous tap), tissue, stool, semen, vaginal swab, Bartholine abscess, Prostatic abscess, Foley's tip from both IPD and OPD by adopting universal safety precautions and fulfilling inclusion criteria. [9]

Isolation of Uropathogen was done by a surface streak procedure on Cysteine Lactose Electrolyte Deficient Agar media (CLED) using calibrated inoculating wire loop. Urine specimens were collected by a clean-catch midstream or catheterization in a sterile container. The inoculated culture plates were aerobically incubated at 37°C for 24-48 hours. Other clinical specimens like body fluids, pus/wound swab, Tissue specimen are streaked over Mac-Conkey, Nutrient Agar media and Sheep blood Agar media followed by aerobic incubation at 37°C for 24-48 hours. While respiratory tract secretions like Sputum, BAL, postbronchoscopy sputum, tracheal secretions are streaked over Nutrient Agar media and Mac-Conkey media at 37°C for 24-48 hours. Antimicrobial sensitivity test was performed over Muller Hinton agar medium by Kirby-Bauer disc diffusion method. [10]

Disc diffusion tests were performed and results were recorded as resistant(R), sensitive(S) and Intermediate (MS). Antibiotics like Ampicillin, Amikacin, Ampicillin/sulbactam, Aztreonam, Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, Cefuroxime, Colistin, Ciprofloxacin, Cotrimoxazole, Cefopera/sulbactam, Cefuroxime, Doripenem, Ertapenem, Gentamicin, Imipenem, Levofloxacin, Meropenem, Nitrofurantoin, Norfloxacin, Ofloxacin, Pipracillin/Tazobactam, Polymixin B, Tobramycin, Tetracycline, Ticarcillin/clavulanic, Teigecyclinne were used against the known control strains ATCC 25922, ATCC 25923 and ATCC 27853 as quality control as per CLSI guidelines. Statistical Package for Social Sciences

(SPSS). Version 22 was used for all the statistical analysis and the results obtained were tabulated and also represented graphically.

Results:

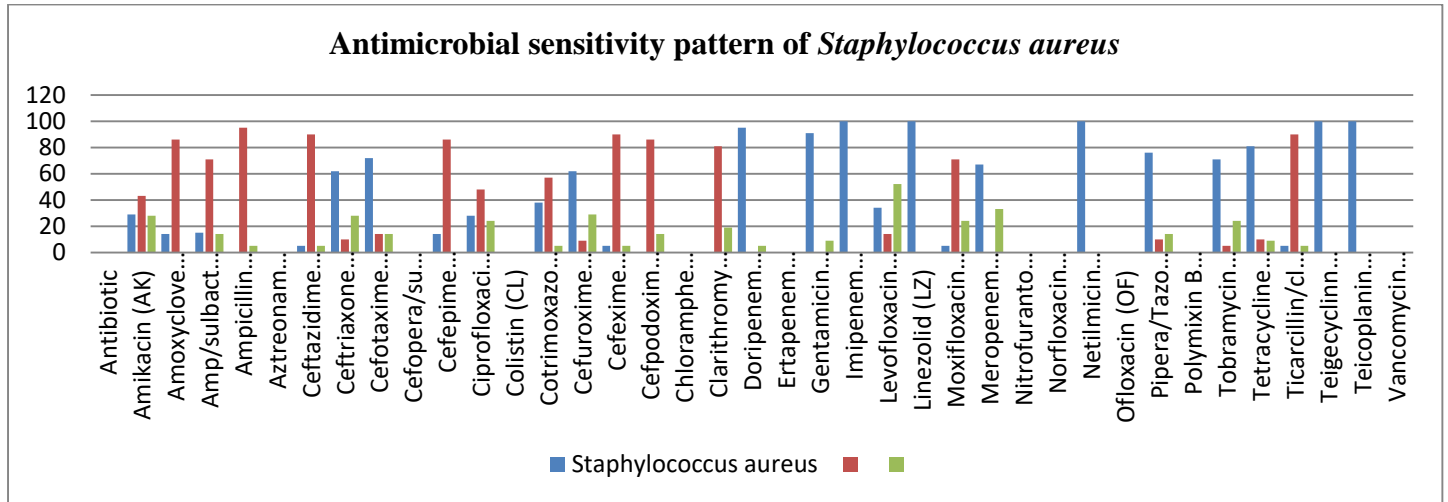


Fig (1): Antimicrobial sensitivity pattern of *Staphylococcus aureus*

Antibiotic	Staphylococcus aureus			Enterococcus spp.			Pseudomonas aeruginosa			Proteus spp.			Acinetobacter spp.			E.coli			Klebsiella pneumoniae		
	S(%)	R (%)	I (%)	S(%)	R(%)	I (%)	S(%)	R (%)	I (%)	S(%)	R (%)	I (%)	S(%)	R (%)	I (%)	S(%)	R (%)	I (%)	S(%)	R (%)	I (%)
Amikacin (AK)	29	43	28				52	31	17	0	33	67	20	67	13	83	14	3	32	58	10
Amoxiclove (AMC)	14	86	0																		
Amp/sulbact (A/S)	15	71	14							33	67	0	10	80	10	3	85	12	8	85	7
Ampicillin (AMP)	0	95	5	0	100	0				0	100	0	0	100	0	0	100	0	5	95	0
Aztreonam (AT)							17	57	26	0	100	0	0	97	3	23	57	20	18	81	1
Ceftazidime (CAZ)	5	90	5				4	87	9	0	100	0	0	100	0	9	72	19	8	85	7
Ceftriaxone (CTR)	62	10	28				17	61	22	0	100	0	0	90	10	27	61	12	25	72	3
Cefotaxime (CTX)	72	14	14				39	17	44	0	0	100	3	87	10	30	65	5	24	71	5
Cefopera/sulbactam (CFS)							61	39	0	33	0	67	14	83	3	26	38	36	18	71	11
Cefepime (CPM)	14	86	0				9	74	17	0	100	0	0	100	0	23	70	7	13	83	4
Ciprofloxacin (CIP)	28	48	24				35	61	4	0	33	67	13	87	0	17	75	8	14	79	7
Colistin (CL)							100	0	0				100	0	0	100	0	0	100	0	0
Cotrimoxazole (COT)	38	57	5							0	100	0	7	87	6	34	66	0	22	74	4
Cefuroxime (CXM)	62	9	29							0	100	0	0	100	0	0	74	26	0	92	8
Cefexime (CFM)	5	90	5							0	100	0	0	100	0	2	81	17	5	92	3

Cefpodoxime (CPD)	0	86	14																		
Chloramphenicol (C)				100	0	0															
Clarithromycin (CLR)	0	81	19																		
Doripenem (DOR)	95	0	5				70	26	4	33	33	34	13	47	40	72	8	20	50	38	12
Ertapenem (ETP)									0	33	33	34	17	73	10	28	38	34	16	63	21
Gentamicin (GEN)	91	0	9	0	100	0	57	43	0	67	33	0	17	73	10	60	21	19	34	62	4
Imipenem (IPM)	100	0	0				87	13	0	100	0	0	80	3	17	88	0	12	92	0	8
Levofloxacin (LE)	34	14	52				48	52	0	0	67	33	14	73	13	23	54	23	13	68	19
Linezolid (LZ)	100	0	0	100	0	0															
Moxifloxacin (MO)	5	71	24																		
Meropenem (MRP)	67	0	33				48	52	0	33	67	0	13	67	20	52	21	28	76	0	24
Nitrofurantoin (NIT)				100	0	0	9	30	61							93	6	1	95	4	1
Norfloxacin (NX)							0	43	57	0	100	0				0	100	0	0	100	0
Netilmicin (NET)	100	0	0				48	43	9												
Ofloxacin (OF)							9	30	61	0	100	0				27	72	1	60	37	3
Pipera/Tazobactam (PIT)	76	10	14				74	17	9	100	0	0	3	83	14	25	22	53	71	24	5
Polymixin B (PB)							100	0	0				100	0	0	100	0	0	100	0	0
Tobramycin (TOB)	71	5	24				52	39	9	0	67	33	27	63	10	51	15	34	93	0	7
Tetracycline (TE)	81	10	9	50	50	0							23	60	17	92	5	3	64	29	7
Ticarcillin/clavunic (TCC)	5	90	5				35	65	0	0	100	0	0	100	0	4	94	2	0	97	3
Teigecycline (TGC)	100	0	0										100	0	0	100	0	0	100	0	0

Teicoplanin (TEI)	100	0	0	100	0	0													
Vancomycin (VA)				0	0	100													

Table (1): Antimicrobial sensitivity pattern of isolated microorganisms from various clinical specimens.

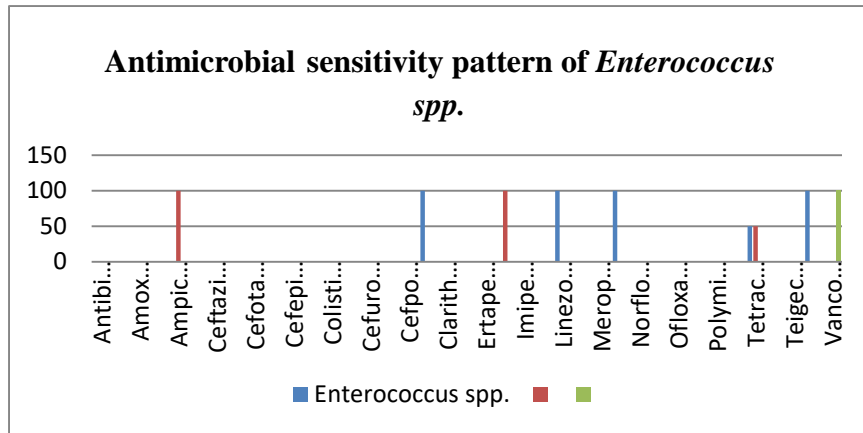


Fig (2): Antimicrobial sensitivity pattern of *Enterococcus* spp.

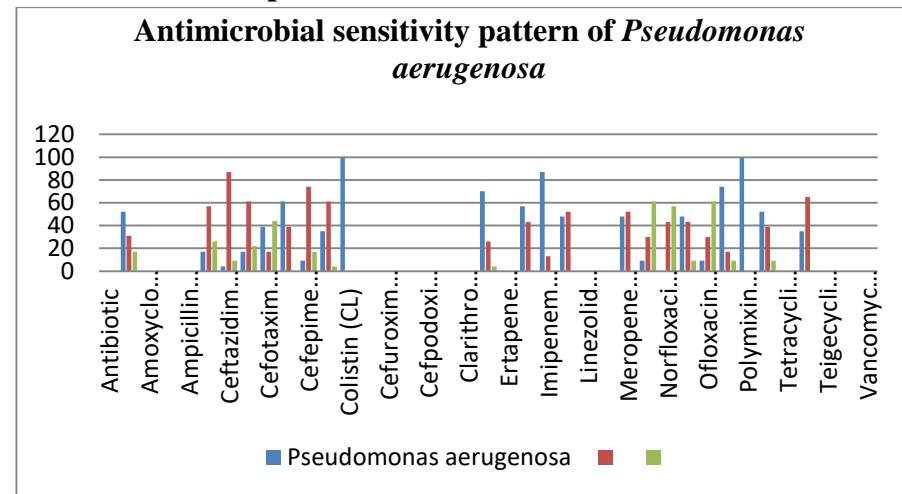


Fig (3): Antimicrobial sensitivity pattern of *Pseudomonas aeruginosa*

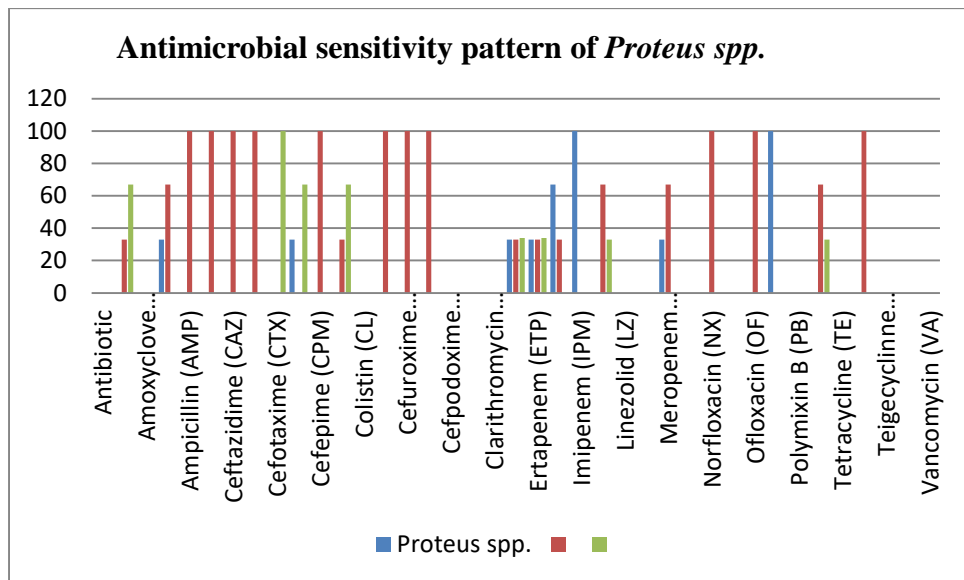


Fig (4): Antimicrobial sensitivity pattern of *Proteus spp.*

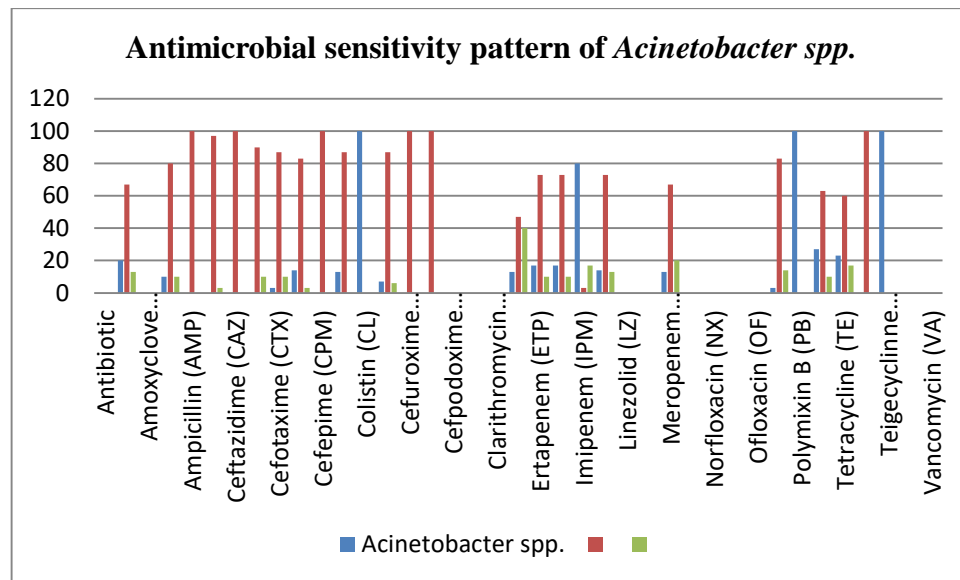


Fig (5): Antimicrobial sensitivity pattern of *Acinetobacter spp.*

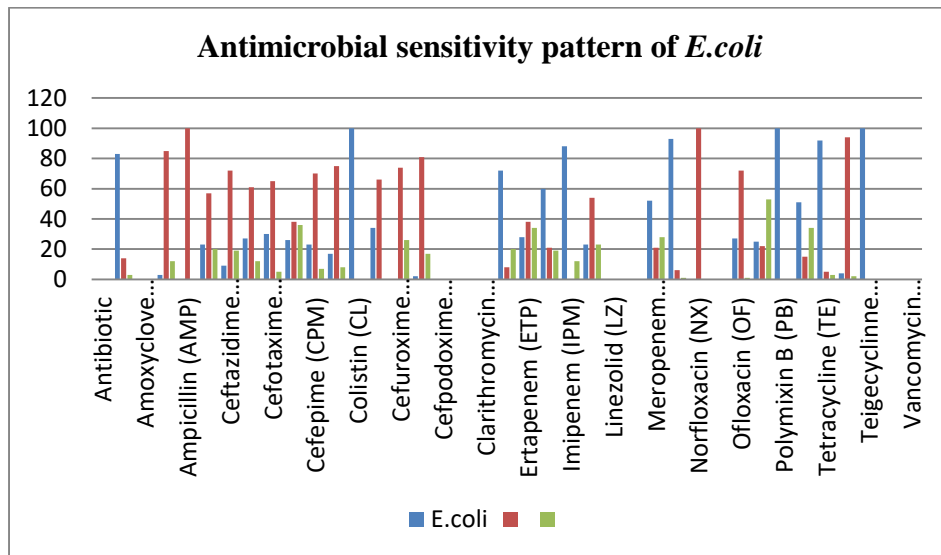


Fig (6): Antimicrobial sensitivity pattern of *E. coli*

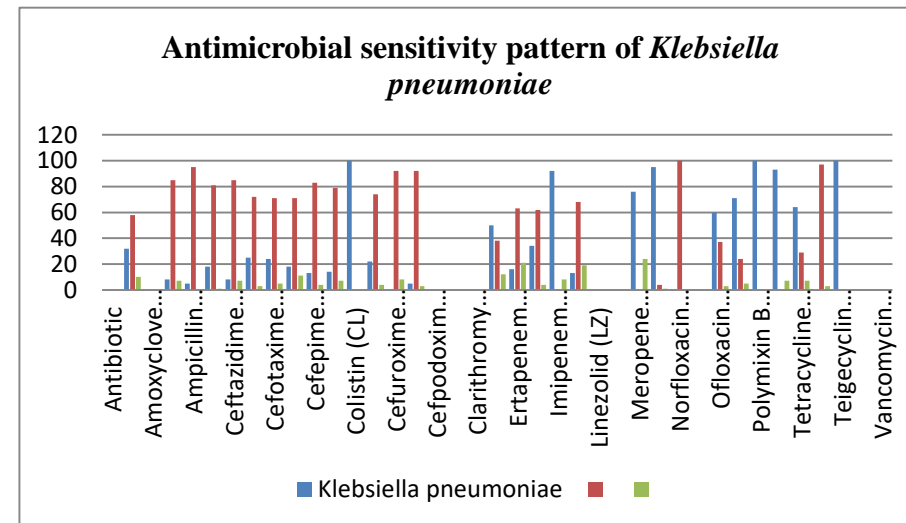


Fig (7): Antimicrobial sensitivity pattern of *Klebsiella pneumoniae*

Discussion:

Several countries across global are facing the occurrence of drug resistant pathogens. Microorganisms are becoming resistant either by modifying their genome or attaining other mode of resistance for existence. [11] The primary cause of resistant microbials is overuse and misuse of antimicrobial medications, drugs that are used empirically or over time in antibiotic therapy, and lack of new medicines. CDC has listed a number of bacteria as critically, severely and with regard to risks. WHO recently published a universal significant list of pathogens with a higher drug-resistant level and classified them in extreme, high and moderate categories of pathogens. [12] Critical includes *Acinetobacter baumannii* (carbapenem-resistant), *Pseudomonas aeruginosa* (carbapenem-resistant) and *Enterobacteriaceae* (carbapenem-resistant, third generation cephalosporin resistant). High includes *Enterococcus faecium* (vancomycin-resistant), *Staphylococcus aureus* (MRSA, vancomycin intermediate and resistant) etc. Medium includes *Streptococcus pneumoniae* (penicillin-non-susceptible), *Haemophilus influenza* (ampicillin resistant) etc. [12-15] E. coli is most predominant and common pathogen that origins injury, UTIs and other microbial infections, is found in this research. The results show that ampicillin exhibited a higher resistance (99.3%) and drugs like Ciprofloxacin and Cefuroxime formerly directed mainly by the Tertiary Health Centre, i.e. (75%) and (80.8%), lead to a complex resistance level. The most potent carbapenem drugs also establish resistance level and middle resistance levels such as Doripenem (7.7%), intermediate (19.9%) and Ertapenem (37.8%), intermediate (34.6%) in some illustrations. Medicines such as cephalosporines of the third generation also lead to resistance. [16] The pathogen thus achieves immunity to antimicrobial agents and results into therapy failure. [17] Similarly, resistance to methicillin, moxifloxacin (71.4 %) and cefexime (90.4 %) was established, while the most sensitive microorganisms were teicoplanin, vancomycin and linezolid, i.e. (100 %). This study showed the rise of resistant pathogens due to the experimental use of broadly used antibiotics. Usually recommended medicines like Cotrimoxazole, Cefuroxime, Ciprofloxacin, ampicillin etc have now dropped due to their less sensitivity for infectious microorganisms. [18-22] CDC studies showed that the definition, medication selection and antibiotic therapy length of treatment were wrong in 30%-50% of the cases. [23] In the growth of resistant bacteria, inaccurately recommended antibiotics play a chief role. [24] To solve this problem, it needs regular antimicrobial surveillance, development and research for proficient drugs and reference rather than emperical prescription of antimicrobial susceptibility test. [25]

Conclusion:

A major concern of public health is the vulnerability of antimicrobial resistance in microorganisms that cause infectious diseases. This results in high healthcare costs and treatment failure. The most frequently prescribed drugs are ineffective because of the pattern of resistance in pathogens in healthcare professionals. The changing trend in the trends of antimicrobial resistance of isolated microorganisms must be controlled, as their limited availability of medicines exceeds the new rates of drug production. Coordinated attempts

must be made to implement new antimicrobial use strategies, to renew research efforts and to take measures to address the AMR crisis.

Conflict of interest:

No conflict of interest.

References:

- [1] Patil, Arun, Kavita Patil, Pankaj Pawar, and Vijay Maheshwari, "Isolation and survey of antibiotic sensitivity in nosocomial infections in North Maharashtra Region." *JAPI*, vol.61, pp. 454-458, Jul.2013.
- [2] Laxminarayan, Ramanan, and Ranjit Roy Chaudhury, "Antibiotic resistance in India: drivers and opportunities for action." *PLoS medicine*, vol.3, March 2016.
- [3] Kaur S., Chauhan, "Development and isolation and characterisation of pathogens from various clinical samples: A step towards prevention of infectious diseases" *J Pharm Sci*, vol.4, pp. 404-409, 2015.
- [4] Duggal, Swati, P. K. Khatri, R. S. Parihar, and Rajat Arora. "Antibiogram of various bacterial isolates from pus samples in a tertiary care centre in Rajasthan." *International Journal of Science and Research*, vol.5, pp.1580-84, May 2015.
- [5] Sangwan, Jyoti, Pooja Singla, Pratibha Mane, Sumit Lathwal, and A. K. Malik, "Prevalence and Antimicrobial Susceptibility Patterns of Aerobic Bacterial isolates from pyogenic wound infections at a tertiary Care Institute in Haryana, India." *International journal of current microbiology and applied sciences*, vol.2, pp. 78-85, 2016.
- [6] Sankarankutty, Jaya, and Soumya Kaup, "Distribution and antibiogram of gram-negative isolates from various clinical samples at a Teaching Hospital, Tumkur." *Blood*, vol.9, pp. 55-5, 2014.
- [7] D'Agata, Erika MC, "Rapidly rising prevalence of nosocomial multidrug-resistant, Gram-negative bacilli: a 9-year surveillance study." *Infection Control & Hospital Epidemiology*, vol.10, pp. 842-846, 2004.
- [8] Sharmin, Sohey, Farhana Alamgir, M. Fahmida, and Ahmed Abu Saleh, "Antimicrobial sensitivity pattern of uropathogens in children." *Bangladesh Journal of Medical Microbiology*, vol.2, pp.18-22, 2009.
- [9] Van Boeckel, Thomas P., Sumanth Gandra, Ashvin Ashok, Quentin Caudron, Bryan T. Grenfell, Simon A. Levin, and Ramanan Laxminarayan, "Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data." *The Lancet Infectious Diseases*, vol. 8, pp. 742-750, 2014.
- [10] Taylor, Peter W, "Alternative natural sources for a new generation of antibacterial agents." *International journal of antimicrobial agents*, vol.3, pp.195-201, 2013.

- [11] Kock, Marleen M., Alessandro N. Bellomo, Nadia Storm, and Marthie M. Ehlers, "Prevalence of carbapenem resistance genes in *Acinetobacter baumannii* isolated from clinical specimens obtained from an academic hospital in South Africa." *Southern African Journal of Epidemiology and Infection*, vol.1, pp. 28-32, 2013.
- [12] World Health Organization, "Antimicrobial resistance global report on surveillance: 2014 summary." World Health Organization, 2014.
- [13] Manikandan, C., and A. Amsath, "from urine samples." *Int. J. Curr. Microbiol. App. Sci*, vol.8, pp. 330-337, 2013.
- [14] Shilpa, K., Ruby Thomas, and Allavarapu Ramyashree, "Isolation and Antimicrobial sensitivity pattern of *Klebsiella pneumoniae* from sputum samples in a tertiary care hospital." *Int J Biomed Adv Res*, vol.2, pp. 53-57, 2016.
- [15] Kollef, Marin H., and Victoria J. Fraser, "Antibiotic resistance in the intensive care unit." *Annals of internal medicine*, vol.4, pp. 298-314, 2001.
- [16] Akram, Mohammed, Mohammed Shahid, and Asad U. Khan, "Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India." *Annals of clinical microbiology and antimicrobials*, vol.1, p. 4, Jan. 2007.
- [17] Tiruneh, Moges, Sisay Yifru, Mucheye Gizachew, Kassie Molla, Yeshambel Belyhun, Feleke Moges, and Mengistu Endris, "Changing trends in prevalence and antibiotics resistance of uropathogens in patients attending the Gondar University Hospital, Northwest Ethiopia." *International journal of bacteriology*, 2014.
- [18] Centers for Disease Control and Prevention, "Office of Infectious Disease Antibiotic Resistance Threats in the United States, 2013." Centers for Disease Control and Prevention, 2013.
- [19] Partridge, Sally R., and Guy Tsafnat, "Automated annotation of mobile antibiotic resistance in Gram-negative bacteria: The Multiple Antibiotic Resistance Annotator (MARA) and database." *Journal of Antimicrobial Chemotherapy*, vol.4, pp. 883-890, 2018.
- [20] Li, Xian-Zhi, Patrick Plésiat, and Hiroshi Nikaido, "The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria." *Clinical microbiology reviews*, vol.2, pp. 337-418, 2015.
- [21] Ruppé, Étienne, Paul-Louis Woerther, and François Barbier. "Mechanisms of antimicrobial resistance in Gram-negative bacilli." *Annals of intensive care*, vol.1, p.21, Dec. 2015.
- [22] Ventola, C. Lee, "The antibiotic resistance crisis: part 1: causes and threats." *Pharmacy and therapeutics*, vol.4, p.277, 2015.
- [23] Hamblin, Michael R, "Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes." *Current opinion in microbiology*, vol.33, pp.67-73, 2016.
- [24] Friedman, N. Deborah, Elizabeth Temkin, and Yehuda Carmeli, "The negative impact of antibiotic resistance." *Clinical Microbiology and Infection*, vol.5, pp.416-422, 2016.
- [25] Cunha, Mirella Alves, Gabriela Lins Medeiros Assunção, Iara Marques Medeiros, and Marise Reis Freitas, "Antibiotic resistance patterns of urinary tract infections in a northeastern Brazilian capital." *Revista do Instituto de Medicina Tropical de São Paulo*, 2016.